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Cadmium-induced genotoxicity detected by the random amplification of polymorphism DNA in the maize seedling roots

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Abstract

In recent years, several plant species have been used as bioindicators, and several tests have been developed to evaluate the toxicity of the environmental contaminants. In this study, the root length and DNA band pattern of root tips in maize (*Zea mays* L.) seedlings were exposed at different concentration of cadmium pollutant (40 and 80 mg L^{-1}). The results indicated that the root length of maize seedlings reduced with an increasing cadmium concentration. A random amplification of polymorphic DNA (RAPD) analysis from the extracted DNA was carried out using twenty three 10-base pair random primers. Eleven primers produced 72 bands between 221-3044 base pairs in gel electrophoresis. DNA damage became evident as the presence and/or absence of DNA fragments in the treated samples compared to the control groups. The number of disappearing bands in profiles increased from 33 at 40 mgL^{-1} of cadmium concentration to 45 after exposure to 80 mg L^{-1} cadmium concentration compared to total bands in control. Three new bands appeared in 40 mgL^{-1} of cadmium concentration but five in 80 mgL^{-1} of cadmium concentration. The results showed that RAPD analysis could be a useful tool for detection of genotoxic effects of cadmium toxicity on plants.

Keywords: Cadmium, Corn, DNA Damage, RAPD Analysis, Root Growth

Introduction

The heavy metal cadmium (Cd) is considered as one of the most dangerous environmental pollutants which usually originates from industrial and agricultural activities such as mining waste disposal and application of pesticides or fertilizers (Agar and Taspinar, 2003). The toxic effects of cadmium have been demonstrated in different plant and animal species. Cd oxidative stress can be involved in Cd toxicity, by either oxygen free radical production (Stohs and Bagchi, 1995; Schutzendubel et al., 2001), or by decreasing the enzymatic and nonenzymatic antioxidants (Sandalio et al., 2001; Fornazier et al., 2002; Cho and Seo, 2004; Surjenru et al., 2007). Cd not only inhibits seed germination, root growth and mitotic index of cells, but also induces damage to different cellular components such as membrane, proteins and DNA (Zhang et al., 1994; Liu et al., 1992; Patra and Panda, 1998; Waisberg et al., 2003; Jimi et al., 2003). Recently, advances in molecular biology have led to using the DNA based techniques (RFLP, RAPD, AFLP, SSR and VNTR) for DNA damage analysis in eco-genotoxicity (Savva, 1996, 1998). RAPD is used extensively for species classification and phylogenetic analysis. A novel application of RAPD method is as biomarker assay to detect DNA damage and mutational events, such as rearrangements point mutation, small insert or deletions of DNA and ploidy changes in cells of bacteria, plants, invertebrate and vertebrate animals (Atienzar et al., 2000). The aim of this study was to detect DNA damage induced by Cd using the RAPD technique. Detection of the genotoxic effect involves comparison of RAPD profiles of the root tip DNA generated by control and treated maize seedlings.

Materials and Methods

Plant materials and treatments

Seeds of *Zea mays* (var KSC.704) were first surface sterilized by using 20-min incubation in 5% (w/v) sodium hypochlorite, followed by three times washing with distilled water. The seeds were then germinated at 24°C and subsequently transferred to pots containing a mixture of sand and perlite (1/1, v/v). The seedlings were grown in a greenhouse under growth conditions of 16 h light and 8 h dark, an average minimum temperature of 18°C, an

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average maximum temperature of 28°C, and the mean humidity of 60%. The heavy metal treatment was performed on 3-days old seedlings exposed to 40 and 80 mgL^{-1} Cd (in the form of $CdN_2O_6.9H_2O_2$) for 7 days.

DNA extraction and RAPD experiment

Approximately 1.5 cm of the seedling root tips of control and treated samples were collected and ground in liquid nitrogen. Total genomic DNA was extracted using modified CTAB assay (Oard and Dronavalli, 1992). The PCR amplification was carried out with twenty three 10-base pair random primers (Eurofins MWG Operon-company) and genomic DNA as the template. PCRs were performed in a reaction mixture of 20 µl containing approximately 80 ng of the genomic DNA dissolving in sterile distilled water, 10X PCR buffer (2µl), 1.5 mM MgCl , 0.25 mM of each dNTP, 2 µl of 10 µM primer and 1 U *Taq* DNA polymerase. The RAPD protocol consisted of an initial denaturing step of 5 min at 94°C, followed by 35 cycles at 94°C for 1min (denaturation), 35°C for 1 min (annealing), and 72°C for 2 min (extension) with an additional extension period of 10 min at 72°C. The PCR amplification products were separated on 1% agarose gel using Tris-Borate-EDTA (TBE) buffer and GeneRuler 100bp DNA ladder (Fermentas, Germany). All the PCR examinations were carried out by Bioer XP thermal.

Statistical analysis and computations

The root growth experiment was conducted as randomized complete block designed with three replicates. Raw data were imported to Microsoft Excel program for calculation and graphic analysis. The SPSS (version 17.0) program was used for analysis of variance and comparison of the means was performed by Duncan's method at $P < 0.05$.

Results

Effect of Cd on root growth

The result (Figure 1) shows the inhibitory effect of Cd treatment on the root length of maize seedlings at two concentrations (40 and 80 mgL^{-1}). The root length decreased 12.42% and 28.32% (significant at $P<0.05$) compared to untreated seedlings.

Effect of Cd on RAPD bands pattern

Out of the 23 random primers tested, only 11 primers gave stable bands (tables 1 and 2). The RAPD fingerprints showed substantial differences between unexposed and exposed seedlings to Cd, with apparent changes in the number and size of the amplified DNA fragments (figures 2 to 5). The number of disappearing bands in 80 mgL^{-1} Cd concentrations was greater than 40 mgL^{-1}.

Disappearing of RAPD bands at 40 mgL^{-1} Cd concentration was occurred with primers OPA-2, OPA-8, OPA-9, OPB-7, OPD-5, OPF-14, OPN-2, OPN-4 and at 80 mgL^{-1} Cd concentration with primers OPA-2, OPA-9, OPD-2, OPD-3, OPD-5, OPF-14, OPN-2, OPN-4. At 40 mgL^{-1} Cd concentration, two new bands (550 and 350bp) appeared with OPD-03 and one new band (472bp) appeared with OPB-07 primer. New bands at 80 mgL^{-1} Cd concentration appeared with OPB-07 primer (one new band; 1073bp), OPN-04 primer (one new band; 679 bp), OPF-14 primer (one new band; 1051bp), OPA-2 primer (one new band; 900 bp) and OPD-5 primer (one new band; 472bp). Eleven primers produced 81 bands between 221-3044 base pairs in gel electrophoresis. The negative control (lane 2 of figure 2) showed that the PCR did not have external contamination.

Discussion

In this study, the root length was severely inhibited by Cd. Changes in the root length of maize seedlings exhibited an inverse relationship with Cd concentration. As Figure 1 shows, exposure of maize seedlings to 80 $mg\,L^{-1}$of Cd concentration has more inhibitory effect on the root length than 40 mgL^{-1} of Cd concentration. These results confirmed that Cd is a toxic agent for plant growth as described by Suzuki (2005). Out of the 23 decamer oligonucleotide primers tested, only 11 primers gave specific and stable results and ten primers of these 11 primers indicated changes in the RAPD profiles following cadmium treatment (Table 1 and Figures 2, 3, 4 and 5). Meanwhile, these primers gave a total of 72 bands ranging from 221-3044 base pairs in gel electrophoresis (Table 2). In this study, DNA damage was shown by RAPD profiles via disappearance or appearance of bands. The number of disappearing RAPD bands in profiles increased from 33 at 40 $mg\,L^{-1}$ of Cd concentration to 45 after exposure to 80 $mg\,L^{-1}$ of Cd concentration, compared to total bands in control. Disappearing bands are likely to be due to changes in oligonucleotide priming sites, originated from rearrangements and less likely from point mutations and DNA damage in the primer binding sites (Nelson et al., 1996; Liu et al., 2005; Enan, 2006; Liu et al., 2009). Three and five new bands were appeared in 40 and 80 $mg\,L^{-1}$ of Cd concentration.

Table 1. Nucleotide sequences of the twenty-three 10-mer primers* used for the random amplification of polymorphism DNA.

Primer	Nucleotide sequence (5′-3′)
OPA-01	CAGGCCCTTC
OPA-02**	TGCCGAGCTG
OPA-03	AGTCAGCCAC
OPA-04	AATCGGGCTG
OPA-07	GAAACCGGTG
OPA-08**	GTGACGTAGG
OPA-09**	GGGTAACGCC
OPA-10**	GTGATCGCAC
OPA-17	GACCGCTTGT
OPA-18	AGGTGACCGT
OPA-20	GTTGCGATCC
OPB-07**	GGTGACGCAG
OPB-10	CTGCTGGGAC
OPC-02	GTGAGGCGTC
OPC-05	GATGACCGCC
OPC-14	TGCGTGCTTG
OPD-02**	GGACCCAACC
OPD-03**	GTCGCCGTCA
OPD-05**	TGAGCGGACA
OPF-14**	GGTGCGCACT
OPN-02**	ACCAGGGGCA
OPN-04**	GACCGACCCA
OPS-09	TCCTGGTCCC

*All primers were provided by Eurofins MWG Operon-company (Ebersberg, Germany).
**Primers which gave optimum bands in the experiment.

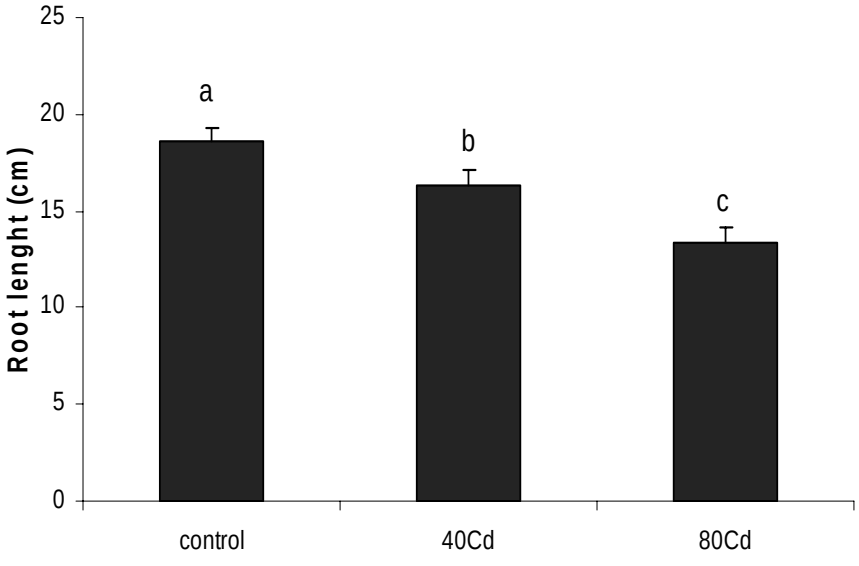

Figure 1. Effects of two Cd concentrations (40 and 80 mg L^{-1}) on root length of the maize seedlings

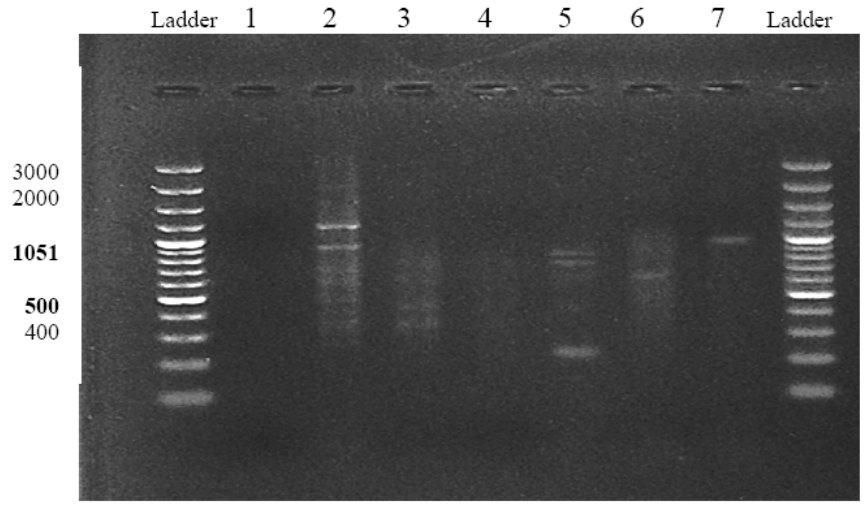

Figure 2. RAPD profiles of genomic DNA from root tips of maize seedlings unexposed and exposed to two Cd concentrations (40 and 80 mg L^{-1}) respectively, using primers OPD-5 (lanes 2, 3, and 4) and OPF-14 (lanes 5, 6, and 7). Lane 1 is negative control. Ladder: 100 bp DNA ladder (100-3000).

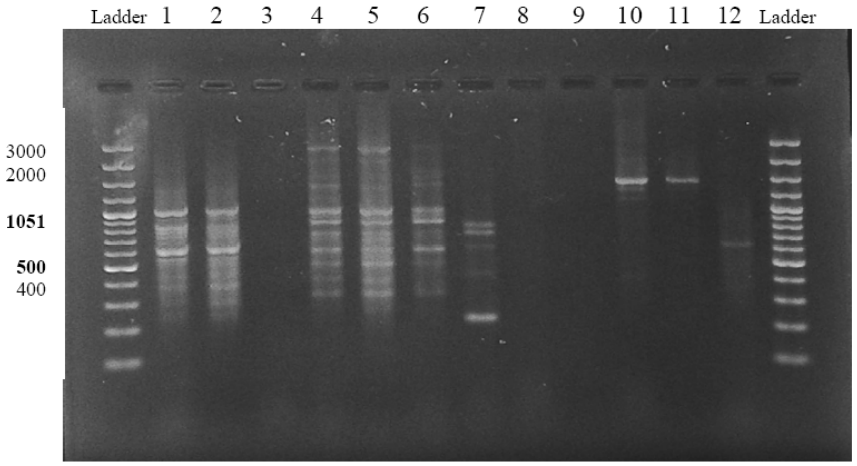

Figure 3. RAPD profiles of genomic DNA from root tips of maize seedlings unexposed and exposed to two Cd concentrations (40 and 80 mg L^{-1}) respectively, using primers OPD-2 (lane 1, 2, and 3), OPD-3 (lane 4, 5, and 6), OPN-2 (lane 7, 8, and 9) and OPN-4 (lane 10, 11, and 12). ladder: 100 bp DNA ladder (100-3000).

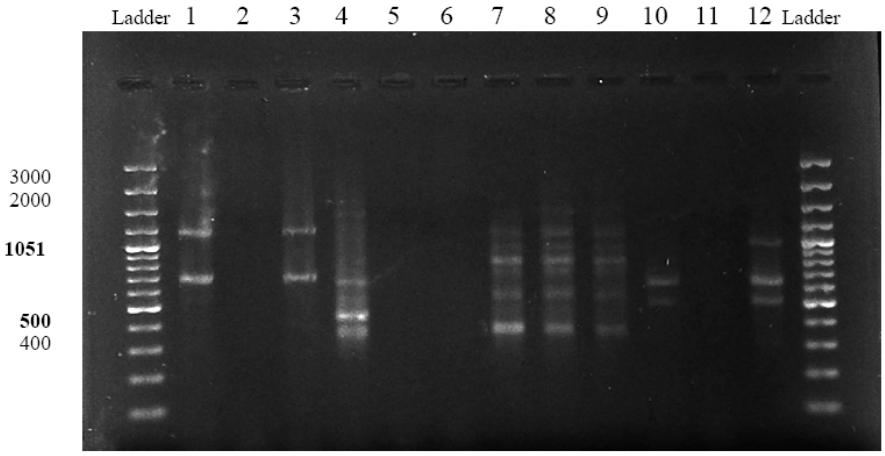

Figure 4. RAPD profiles of genomic DNA from root tips of maize seedlings unexposed and exposed to two Cd concentrations (40 and 80 mg L^{-1}) respectively, using primers OPA-8 (lane 1, 2, and 3), OPA-9 (lanes 4, 5, and 6), OPA-10 (lanes 7, 8, and 9) and OPB-7 (lanes 10, 11, and 12). ladder: 100 bp DNA ladder (100-3000).

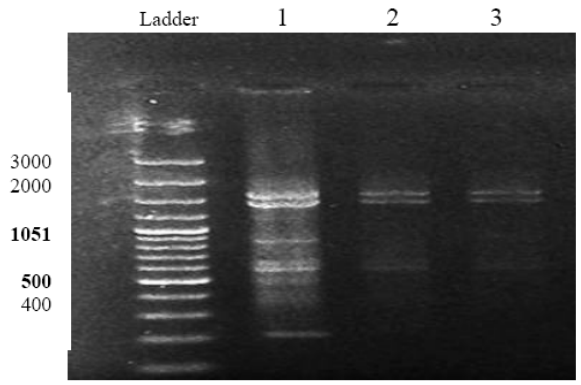

Figure 5. RAPD profiles of genomic DNA from root tips of maize seedlings unexposed and exposed to two Cd concentrations (40 and 80 mg L^{-1}) respectively, using primers OPA-2 (lanes 1, 2, and 3). ladder: 100 bp DNA ladder (100-3000).

Table 2. Molecular sizes (bp) of appeared and disappeared bands by random primers using PhotoCap software

	Treatments				
Primers names	Total bands in control	40 (mgL^{-1}) Cd concentration		80 (mgL^{-1}) Cd concentration	
		Appearance of new bands	Disappearance of control bands	Appearance of new bands	Disappearance of control bands
OPA-2	1679, 1500, 1205, 874, 657, 603, 513, 221		1205, 874, 513, 221	900	1205, 874, 513, 221
OPA-8	1197, 717		1197, 717		No band disappeared
OPA-9	1282,1200,873, 690, 582, 470, 382		1282,1200,873, 690, 582, 470, 382		1282,1200, 873, 690, 582, 470, 382
OPA-10	1631, 1194, 1060, 868, 786, 600, 505, 396		No band disappeared		No band disappeared
OPB-7	690, 547		690, 547	1073	No band disappeared
OPD-2	1366, 1078, 960, 844, 626, 568, 463, 400, 316, 262		No band disappeared		1366, 1078, 960, 844, 626, 568, 463, 400, 316, 262
OPD-3	3044, 2458, 1773, 1500, 1249, 1112, 936, 788, 647, 548, 453, 357	550, 350	No band disappeared		2458, 453
OPD-5	2234, 1243, 1000, 805, 729, 643, 521, 432, 373	472	2234, 1243, 1000, 729, 521, 432	472	2234, 1243, 1000, 805, 729, 643, 521, 373
OPF-14	908, 790, 667, 462, 243		908, 790, 462, 243	1051	908, 790, 667, 462, 243
OPN-2	891, 809, 679, 476, 252		891, 809, 679, 476, 252		891, 809, 679,476, 252
OPN-4	1500, 1446, 1249, 460		1446, 1249, 460	679	1500, 1446, 1249, 460

Structural changes or some changes in DNA sequence due to mutation and/or large deletions (bringing two pre-existing annealing sites closer) create in new priming sites. Previous studies had shown that changes in DNA fingerprint offer a useful biomarker assay in ecotoxicology (Savva, 1996; Savva, 1998). Cd could induce DNA damage such as single- and double-strand breaks, modified bases, abasic sites, DNA-protein crosslink, oxidized bases and even bulky adducts in organisms (Hamada et al., 1997; Aust and Eveleigh, 1999; Bisova et al., 2003; Waisberg et al., 2003; Atesi et al., 2004; Hsiao and Stapleton, 2004; Jimi et al., 2004; Becher et al., 2004; Liu et al., 2005, 2009 and Cencki et al., 2009). Our finding support this claim that DNA polymorphisms detected by RAPD can be considered as a powerful biomarker assay for detection of the genotoxic effects of environmental pollutants like heavy metals. As a tool in risk assessment, the RAPD assay can be used in characterization of Cd hazard in soil. The RAPD-PCR based assay is fast, reliable and easy to conduct in any laboratory for assessment of environmental hazardous metals on plants.

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References

1- Agar G. and Taspinar M. S. (2003) Effect of calcium, selenium and zinc on cadmium induced chromosomal aberration in root of *Secale cereale*. Fresenius Environmental Bulletin 12: 1471-1475.

2- Atienzar F. A., Venier P., Jha A. N. and Depledge M. H. (2002) Evolution of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage and mutations. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 521: 151-163.

3- Atesi I., Suzen H. S., Aydin A. and Karakaya A. (2004) The oxidative DNA base damage in tests of rats after intraperitoneal cadmium injection. Biometals 17: 371-377.

4- Aust A. E. and Eveleigh J. F. (1999) Mecanism of DNA oxidation. Proc Soc Exp Biol Med, 222: 246-252.

5- Becher M., Talke I. N., Krall L. and Kramer U. (2004) Cross species microarray transcript profiling reveals high constitutive expression of metal homeostasis gene in shoots of the zinc hyperaccumulator Arabidopsis halleri. Plant Journal 37: 251-268.

6- Bisova K., Hendrychova J., Cepak V. and Zachleder V. (2003) Cell growth and division processes are differentially sensitive to cadmium in Scenedesmus quadricauda. Folia Microbiologica 48: 805-816.

7- Cenkci S., Yildiz M., Cigerci I., Konuk M. and Bozdag A. (2009) Toxic chemicals-induced gentoxicity detected by random amplified polymorphic DNA (RAPD) in bean (*Phaseolus vulgaris* L.) seedlings. Chemosphere 76: 900-906.

8- Cho U. and Seo N. (2004) Oxidative stress in Arabidopsis thaliana exposed to cadmium is due to hydrogen peroxide accumulation. Plant Science 168: 113-120.

9- Enan M. R. (2006) Application of random amplified polymorphic DNA (RAPD) to detect the genotoxic effect of heavy metals. Biotechnology and Applied Biochemistry 43: 147-154.

10- Fornazier R., Ferreira R., Vitoria A., Molina S., Lea P. and Azevedo R. (2002) Effects of cadmium on antioxidant enzyme activities in sugar cane. Biologia Plantarum 45(1): 91- 97.

11- Hamada T., Tanimoto A. and Sasaguri Y., (1997) Apoptosis induced by cadmium. Apoptosis 2: 359-367

12- Hsiao C. J. and Stapleton S. R. (2004) Characterization of Cd-induced molecular events prior to cellular damage in primary rat hepatocytes in culture: activation of the stress activated signal protein JNK and transcription factor AP-1. Journal of Biochemical and Molecular Toxicology 18: 133-142

13- Jimi S., Uchiyama M., Takaki A., Suzumiya J. and Hara S. (2004) Mechanism of cell death induced by cadmium and arsenic . Annals of the New York Academy of Sciences 1011: 325-331.

14- Liu D. H., Jiang W. S. and Li M. X. (1992) Effect of cadmium on root growth and cell division of Allium cepa. Acta Scientiae Circumstantiae 12: 339- 406.

15- Liu W., Li P., Qi X., Zhou Q., Zheng L., Sun T. H. and Yang, Y. S. (2005) DNA changes in barely (*Hordeum vulgare*) seedlings induced by cadmium pollution using RAPD. Chemosphere 61: 158-167.

16- Liu W., Yang Y. S., Li P. J., Zhou Q. X., Xie L. J. and Han Y. P. (2009) Risk assessment of cadmium-contaminated soil on plant DNA damage using RAPD and physiological indices. Journal of Hazardous Materials 161: 878-883.

17- Nelson J. R., Lawrence C. W. and Hinkle D. C. (1996) Thymine-thymine dimmer bypass by yeast DNA-polymerase-zeta. Science 272: 1646-1649.

18- Oard J. H., Dronavalli S. (1992) Rapid isolation of rice and maize DNA for analysis by random-primer PCR. Plant Molecular Biology Reporter 10: 236-241.

19- Patra J., Panda B. B. (1998) A comparison of biochemical responses to oxidative and metal stress in seedlings of barely, *Hordeum vulgar* L. Environmental Pollution 101: 99-105.

20- Sandalio L., Dalurzo H., Gomez M., Romerio P. and Riola D. (2001) Cadmium- induced changes in growth and oxidative metabolism of pea plants. Journal of Experimental Botany 52: 2115-2126.

21- Savva D., (1996) DNA fingerprints as a biomarker assay in ecotoxicology. Toxicology and Ecotoxicology News 3: 110-114.

22- Savva D., (1998) Use of DNA fingerprinting to

detect genotoxic effects. Ecotoxicology and Environmental Safety 41: 103-106.

23- Schutzendubel A., Schwanz P., Teichmann T., Gross K., Heyser R. L., Godbold D. L., Polle A., (2001) Cadmium-induced changes in antioxidative systems, hydrogen peroxide content, and differentiation in Scots pine roots. Plant Physiology 127: 887-898.

24- Stohs S. J. and Bagchi D. (1995) Oxidative mechanisms in the toxicity of metal ions. Free Radical Biology and Medicine 18: 321-336.

25- Surjendu K., Jayashree D., Sanjukta P. and Debasmite P. (2007) Changes in the antioxidative enzyme activities and lipid peroxidation in wheat seedlings exposed to cadmium and lead stress. Brazilian Journal of Plant Physiology 19: 219-229.

26- Suzuki N. (2005) Alleviation by calcium of cadmium-induced root growth inhibition in Arabidopsis seedlings. Plant Biotechnology 22: 19-25.

27- Waisberg M., Joseph P., Hale B. and Beyersman D. (2003) Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology, 192: 95-117.

28- Zhang X. Y. and Yang X. L., (1994) The toxic effects of cadmium on cell division and chromosomal morphology of *Hordeum vulgare*. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 312:121-126..