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*Full Length Research Paper*

# Investigation of DNA changes in wheat (*Triticum aestivum* L.) induced by cadmium using random amplified polymorphic DNA (RAPD) analysis

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**In recent years, many plants have been used as bioindicators or living indices and several experiments have been conducted to evaluate the genotoxic effects of environmental pollution on plant species. Plants such as Arabidopsis and barley have been used as biological indicators by several researchers and have been able to identify DNA changes using molecular markers. This study was conducted on wheat as an important cereal that provides human daily food and has an important role in human health. The purpose of study was to evaluate the effect of different concentrations of cadmium on amounts of the soluble proteins, investigation of growth and possible changes to the structure of DNA using random amplified polymorphic DNA (RAPD) marker. In the present study, wheat seedlings were used for detection of genotoxic effects of cadmium contamination in the range of 0 to 120 $mgl^{-1}$. The limiting effects of cadmium on length of root and plant height and total soluble proteins in root were quite evident by increase of cadmium concentration. Change in DNA was observed using RAPD marker as there was change in number of bands, present or absent of bands at the range of above 5 $mgl^{-1}$ cadmium concentration. In the previous studies conducted by several researchers, variation in DNA bands was reported at levels above 30 $mgl^{-1}$, whereas in the present study, band variation was observed at the concentration of 5 $mgl^{-1}$. This study also showed that DNA stability is highly affected by cadmium pollution at >5 $mgl^{-1}$ which was identified by RAPD markers.**

**Key words:** Bioindicator, cadmium, genotoxic, random amplified polymorphic DNA (RAPD) marker, *Triticum aestivum* L.

## INTRODUCTION

In the last several decades, cadmium (Cd) has been used extensively as materials and/or intermediates in the chemical industry and agriculture for electroplating, metallurgy, painting, plastic production, etc., and is being released into the biosphere, and largely found in the water and soil (Baryla et al., 2001; Liu et al., 2005;

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Shahrtash et al., 2010). Through food chain, Cd accumulation could pose a direct threat to human health and cause diseases such as hypertension, angiopathy, kidney and bone function decay (Liu et al., 2005). The toxic and genotoxic effects of cadmium have been demonstrated in different plant and animal species and it has been shown that cadmium caused change in DNA and cellular structures (Conte et al., 1998; Liu et al., 2005; Shahrtash et al., 2010). However, the molecular mechanism responsible for the genotoxicity of cadmium remains unclear. It has been suggested that it may involve direct interaction of $Cd^{2+}$ with DNA through the binding of Cd at G, A and T bases (Valverde et al., 2001; Hossain and Huq, 2002; Anastassopoulou, 2003). Furthermore, recent studies indicate that Cd acts as a mutagen primarily by direct inhibition of an essential DNA mismatch repair, resulting in a high level of genetic instability (Andrea, 1994; Jin et al., 2003). Cadmium cellular toxicity and genotoxicity may also be mediated indirectly; cells under oxidative stress display various dysfunctions due to lesions caused by reactive oxygen species (ROS, e.g. $O_2^-$, $H_2O_2$ and $OH^-$) to lipids, proteins and DNA (Waisberg et al., 2003; Joseph, 2009). Advantages of measuring effects of genotoxic chemicals directly on DNA are mainly related to the sensitivity and short response time. Recently, advances in molecular biology have led to the development of a number of selective and sensitive assays for DNA analysis in eco-genotoxicology. DNA based techniques [RFLP, QTL, random amplified polymorphic DNA (RAPD), AFLP, SSR and VNTR] are used to evaluate the variation at the DNA sequence level. RAPD of these techniques can be used to detect genotoxicity and differences in RAPD profiles can clearly be shown when comparing DNA fingerprints from untreated and treated individuals to genotoxic agents (Savva, 1996; Conte et al., 1998; Theodorakis and Bickham, 2001; Enan, 2006; Atienzar and Jha, 2006; Liu et al., 2007; Cenkci et al., 2009). 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 wheat seedlings.

## MATERIALS AND METHODS

### Plant material, growth and total soluble protein level test of seedlings

Seeds of Cascogen wheat cultivar provided by Seed and Plant Improvement Institute of Mashhad, were sterilized by using incubation in 5% (w/v) sodium hypochlorite, soaked in distilled water, incubated for three days in a dark growth chamber at temperature of 18 ± 1°C and germinated to primary roots of 1 cm long. Uniformly 100 germinated seeds were selected and transferred to Petri pots (length 18 cm, width 10 cm and height 7 cm) containing 0.5 kg sandy soil of 1 to 3 mm (washed with 1 N HCl) with 200 ml of distilled water or test solution. The growth inhibition test was performed with the above wheat plantlets exposed to 0, 5, 10, 15, 20, 30, 60, 90, 120 $mgl^{-1}$ Cd (in the form of $CdCl_2.H_2O$ with purity of 99.9% (Merk, Germany) for nine days, respectively. Petri pots were incubated in a growth chamber at temperature of 20 ± 1°C and a 16-h-day-8-h-night photoperiod. After nine days of incubation, the root and shoot length was measured using a ruler and total soluble protein level of root-tips in plantlets were measured with Bradford method (1976) by using nanodrop (Thermo scientific nanodrop 2000 Spectophotometry, U.S.A). The data were expressed as mean ± HSD. Inhibitory rate (IR, %) of the above indices was calculated using the following formula: IR = (1- x/y) × 100, where x and y are the average values detected in the control and each sample treated, respectively. Three replicate were set up for each treatment and comparison experiments were carried out as duplicate. The differences between mean values of treated plantlets and the controls were statistically investigated using HSD tests.

### DNA extraction and RAPD procedures

After nine days of growth, approximately 1 cm root tips of seedlings were collected, ground in liquid nitrogen, and total genomic DNA was extracted using DNA purification kit (Germany, 5 prime). Purified DNA concentration and integrity of total genomic DNA in each sample were estimated by nanodrop (Thermo scientific nanodrop 2000 Spectophotometry., U.S.A). The PCR amplification was carried out with 15 10-base pair random primers (Bioneer-company) and genomic DNA as the template (Table 1). PCR were performed in a reaction mixture of 25 µl containing approximately 75 ng of the genomic DNA dissolving in sterile TE, 10X PCR buffer (2/5 µl), 2 mM $MgCl_2$, 0.2 mM of each dNTP, 2 µl of 10 µM primer and 1 U Taq DNA polymerase. Amplifications were performed in a DNA thermocycler (Biometra., U.S.A) programmed for 4 min at 95°C (initial denaturing step), 35 consecutive cycles each consisting of 4 min at 94°C (denaturing), 1 min at 34°C (annealing), 2 min at 72°C (extension), and followed by 1 cycle for 10 min at 72°C (final extension step). Negative controls with water, without any template DNA, were always included to monitor contamination. PCR reaction products were mixed with one-sixth volume of gel loading buffer (Fermentas, Germany), and then separated on 1.1% agarose gel using Tris-Borate- EDTA (TBE) buffer and GeneRuler DNA ladder (Fermentas, Germany). DNA bands were stained with ethidium bromide, visualized and photographed under UV light. The size and intensity of each amplification product was automatically estimated using the UV-Transilluminator image analyzer system.

### RAPD profile and data analysis

All amplifications were repeated twice in order to confirm the reproducible amplification of scored fragments. Only reproducible and clear amplification bands were scored for the construction of the data matrix. Polymorphism observed in RAPD profiles included disappearance and/or appearance and variation in band intensity when comparison with untreated control treatments were evaluated (Liu et al., 2005; Cenkci et al., 2009). Change in soluble protein level and growth rate values were calculated as a percentage of their control (Liu et al., 2005).

### Statistical analysis and computations

The growth index experiments and total soluble protein content was calculated according to randomized complete block designed with three replicates. Raw data were imported to Microsoft Excel 2010 program for calculation. The SPSS (Version 17.1) program was used for analysis of variance and comparison of the means was performed by HSD's method at $P< 0.05$.

**Table 1.** Sequences and temperature (°C) of the 15 primers used in this experiment.

| Number of primer | Sequences of primer | Temperature (°C) |
|---|---|---|
| A.z 1 | 5´-CAATCGCCGT-3´ | 32 |
| A.z 2 | 5´-TCGGCGATAG-3´ | 32 |
| A.z 3 | 5´-GAAGCGCGAT-3´ | 32 |
| A.z 4 | 5´-GCTGCGTGAC-3´ | 34 |
| A.z 5 | 5´-CGACTCACAG-3´ | 32 |
| A.z 6 | 5´-CCACCCGAGG-3´ | 36 |
| A.z 7 | 5´-TACCGTCGGA-3´ | 32 |
| A.z 8 | 5´-CGACAGTCCC-3´ | 34 |
| A.z 9 | 5´-CTTGACGGGG-3´ | 34 |
| A.z 10 | 5´-TCCCTCGTGC-3´ | 34 |
| A.z 11 | 5´-CAGGCCCTTC-3´ | 34 |
| A.z 12 | 5´-AATCGGGCTG-3´ | 32 |
| A.z 13 | 5´-GTCCCGACGA-3´ | 34 |
| A.z 14 | 5´-ACGGTACCAG-3´ | 32 |
| A.z 15 | 5´-AATCGGGCTG-3´ | 32 |

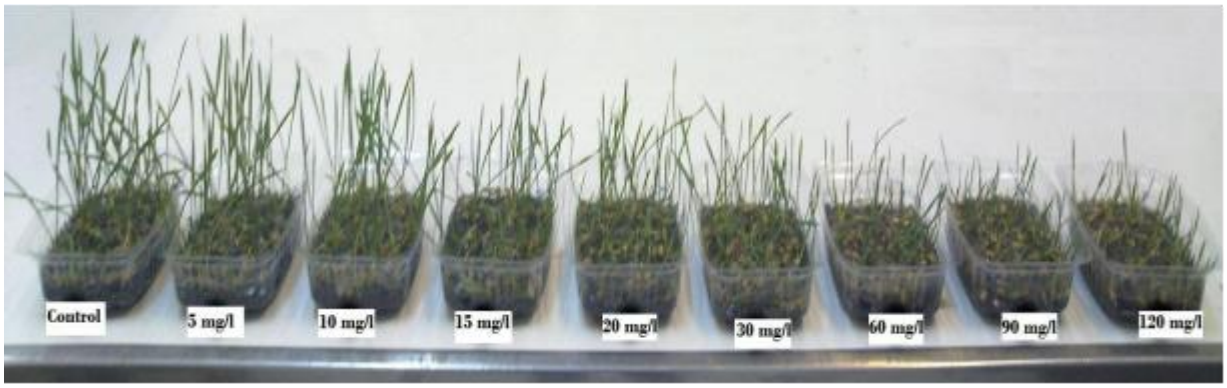


**Figure 1.** Effect of Cd on wheat seedling length after nine days of treatment.

## RESULTS

### Effect of Cd on root and shoot growth in wheat seedlings

Cadmium treatment showed toxic effect on seedling growth and root growth of wheat seedlings in all treatment as compared to the control (Figure 1). Seedling and root length of wheat were significantly ($p<0.05$) decreased at 10, 15, 20, 30, 60, 90, 120 $mgl^{-1}$ of cadmium treatment as compared to untreated seedlings. The inhibitory rate of root and shoot growth is given in Table 2. Toxicity effect of cadmium on plant has been previously described in few studies (Shafiq et al., 2008; Liu et al., 2005).

### Effect of Cd on total soluble protein content of root tips of wheat seedlings

The data for total soluble protein level in root tips of seedlings are shown in Table 3. Total soluble protein levels in seedlings decreased at 5, 10, 15, 20, 30, 60, 90, and 120 $mgl^{-1}$ Cd, but significant ($P<0.05$) reduction was obtained at concentrations above 10 $mgl^{-1}$ Cd, and inhibited substantially respectively when compared with control plantlets. Results show that there was a negative correlation between Cd concentration and total soluble protein content in wheat seedlings, with a correlation coefficient of ($r^2$) 0.825.

### DNA extraction

Since extracted genomic DNA was in varied concentration, it is needed to equal the concentration of DNA for our experiment. So genomic DNA concentration was equaled by using nanodrop and separated on agarose gel. Nanodroping and electrophoresis data showed that purity grade of DNA is suitable for RAPD. The integrity of the genomic DNA extracted is shown in Figure 2.

**Table 2.** Effect of Cd on root and shoot length of wheat seedlings after 9 days of treatment.

| Cd concentration ($mgl^{-1}$) | Root | | Plant length | |
|---|---|---|---|---|
| | Root length (cm) | Inhibitory rate (%) | Plant length (cm) | Inhibitory rate (%) |
| 0 | 17.5[a] | - | 13.2a | - |
| 5 | 17[a] | 2.8 | 13a | 1.5 |
| 10 | 15.9[b] | 9.1 | 12.1b | 8.3 |
| 15 | 14.75[c] | 15.7 | 11.3b | 14.3 |
| 20 | 13.3[d] | 24 | 9.3c | 29.5 |
| 30 | 12.25[e] | 30 | 9.3d | 29.5 |
| 60 | 10.3[f] | 41.1 | 8.6e | 34.8 |
| 90 | 9[g] | 48.5 | 8.3f | 52.2 |
| 120 | 7.5[h] | 57.1 | 3.8g | 71.2 |

*Average comparison done by using HSD test ($p<0.05$).

**Table 3.** Effect of Cd on total soluble protein content of root tips seedlings after 9 days of treatment.

| Cd concentration ($mgl^{-1}$) | Total soluble protein of root-tips | |
|---|---|---|
| | Soluble protein level (ng/µl) | Inhibitory rate (%) |
| 0 | 444.37[a]* | - |
| 5 | 439.97[a] | 1.11 |
| 10 | 408.93[b] | 8.33 |
| 15 | 378.4[c] | 14.93 |
| 20 | 360.27[d] | 19.10 |
| 30 | 328.77[e] | 26.15 |
| 60 | 301.77[f] | 32.47 |
| 90 | 290.97[g] | 34.98 |
| 120 | 265.5[h] | 40.24 |

*Average comparison done by using HSD test ($p<0.05$).

## RAPD profile analysis

Aimed at verifying the genetic effect of Cd tested, the RAPD analysis was performed on DNA extracted from the roots. Fifteen (15) 10mer oligonucleotide primers of 70 to 75% GC content were utilized for screening the wheat genome for changes, whilst only 13 primers generated specific and stable results. The total number of bands was 80 for untreated control treatments. All 13 primers produced same RAPD profiles for the untreated roots seedlings. On the other hand, RAPD profiles showed substantial differences between untreated control and treated seedlings with apparent changes (disappearance and/or appearance) in the number and size of amplified DNA fragments for different primers. The changes in RAPD profiles were summarized for wheat roots of treated seedlings in comparison to their controls (Tables 4 and 5). The maximum change in RAPD profiles (disappearance b and/or appearance a) was obtained in 120, 90, 60, 20, 15 and 30 $mgl^{-1}$ of Cd concentration, respectively, when compared with control plantlets. Also, maximum change in band intensity (decrease d or increase c) was obtained in 90, 120, 60, 15, 20 and 30 $mgl^{-1}$ of Cd, as compared to untreated seedling. In this experiment, change in band intensity (decrease and increase) greatly occurred in high Cd concentrations. For Az.1 primer, disappearances of bands were more evident in 120, 30 and 20 $mgl^{-1}$ concentration of Cd (Figure 3). With Az.2 and Az.4 primers, no change was observed in RAPD profiles (Figure 3). With Az.3 primer, new bands appeared in 20, 30, 60, 90 and 120 $mgl^{-1}$ of Cd concentration (Figure 3). For Az.6, Az.5 and Az.13 primers, disappearance of bands occurred in 5 $mgl^{-1}$ of Cd concentration (Figure 3). Disappearance of bands was also detected in 15, 20, 60,90 and 120 $mgl^{-1}$ with Az.7 primer (Figure 3). Disappearance and decrease in band was observed in 15, 20 and 30 $mgl^{-1}$, and increase in band intensity was detected in 60, 90 and 120 $mgl^{-1}$ (Figure 3). With Az.12 primer, band intensity was only an appearance of DNA change (Table 5) (Figure not shown). Appearance of new bands in low molecular weight was obtained in Az.13 profiles above 15 $mgl^{-1}$ Cd (Table 5)

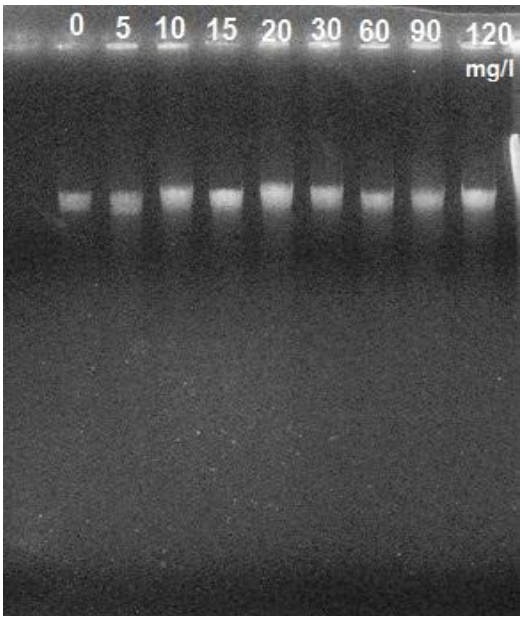


**Figure 2.** Genomic DNA of about 100 ng extracted by using DNA purification kit (Germany, 5 PRIME) in root-tips of wheat.

**Table 4.** Changes in number of appearing and disappearing bands in control, and of polymorphic bands and varied bands in Cd-contaminated wheat seedlings.

| Number of primer | Cd concentration ($mgl^{-1}$) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 5 | | 10 | | 15 | | 20 | | 30 | | 60 | | 90 | | 120 | | |
| | | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b | |
| A.Z1 | 13 | 0 | 2 | 0 | 2 | 0 | 1 | 0 | 7 | 0 | 6 | 0 | 0 | 0 | 2 | 0 | 7 | |
| A.Z2 | 3 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | |
| A.Z3 | 5 | 0 | 1 | 0 | 0 | 0 | 1 | 2 | 0 | 2 | 1 | 2 | 2 | 3 | 0 | 3 | 0 | |
| A.Z4 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| A.Z5 | 4 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | |
| A.Z6 | 10 | 0 | 1 | 0 | 2 | 0 | 1 | 0 | 1 | 0 | 2 | 0 | 0 | 0 | 3 | 0 | 3 | |
| A.Z7 | 5 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | |
| A.Z8 | 6 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | |
| A.Z9 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | |
| A.Z10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| A.Z11 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| A.Z12 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| A.Z13 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 2 | 1 | 1 | |
| A.Z14 | 8 | 0 | 0 | 1 | 0 | 4 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 6 | 0 | 6 | 0 | |
| A.Z15 | 7 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | |
| Total bands | 80 | 1 | 5 | 3 | 1 | 7 | 9 | 4 | 13 | 4 | 10 | 5 | 14 | 12 | 9 | 12 | 15 | |
| a + b | | 6 | | 4 | | 16 | | 17 | | 14 | | 19 | | 21 | | 27 | | |

a, Indicates appearance of new bands; b, disappearance of normal bands; a + b, polymorphic bands.

(Figure not shown). The number of disappearance of RAPD bands was more at two Cd concentrations (90 and 120 $mgl^{-1}$) and appearance of new bands was greater at three Cd concentrations (15, 90 and 120 $mgl^{-1}$) for Az.14 primer. Absent of bands was particularly obvious for wheat exposed to 60, 90 and 120 $mgl^{-1}$ Cd for primer Az.15 (Table 4) (Figure not shown). Decrease or increase in bands intensity generated by different primers is given

**Table 5.** Changes of total bands intensity in control, and polymorphic bands and varied bands in Cd-contaminated wheat seedlings.

| Number of primer | Cd concentration ($mgl^{-1}$) | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 5 | | 10 | | 15 | | 20 | | 30 | | 60 | | 90 | | 120 | |
| | | c | d | c | d | c | d | c | d | c | d | c | d | c | d | c | d |
| A.Z1 | 13 | 1 | 0 | 2 | 2 | 2 | 3 | 1 | 0 | 5 | 0 | 4 | 1 | 3 | 1 | 2 | 0 |
| A.Z2 | 3 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 2 | 0 |
| A.Z3 | 5 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| A.Z4 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A.Z5 | 4 | 0 | 0 | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 1 |
| A.Z6 | 10 | 2 | 0 | 0 | 0 | 4 | 0 | 5 | 0 | 4 | 0 | 4 | 0 | 5 | 0 | 5 | 0 |
| A.Z7 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| A.Z8 | 6 | 1 | 0 | 1 | 0 | 2 | 0 | 4 | 0 | 2 | 0 | 2 | 0 | 2 | 0 | 5 | 0 |
| A.Z9 | 7 | 2 | 0 | 0 | 0 | 2 | 0 | 0 | 4 | 0 | 3 | 4 | 0 | 0 | 4 | 4 | 0 |
| A.Z10 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A.Z11 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| A.Z12 | 7 | 0 | 0 | 5 | 1 | 5 | 0 | 5 | 1 | 5 | 1 | 5 | 1 | 5 | 1 | 1 | 0 |
| A.Z13 | 3 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 |
| A.Z14 | 8 | 1 | 0 | 1 | 0 | 2 | 2 | 6 | 0 | 6 | 0 | 4 | 0 | 0 | 7 | 0 | 7 |
| A.Z15 | 7 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 1 | 0 | 1 | 4 | 0 | 4 | 0 | 4 | 0 |
| Total bands | 80 | 8 | 1 | 15 | 4 | 27 | 7 | 23 | 8 | 23 | 6 | 29 | 4 | 21 | 17 | 25 | 8 |
| a + b | | 9 | | 19 | | 34 | | 31 | | 29 | | 33 | | 38 | | 33 | |
| a + b + c + d | | 15 | | 23 | | 50 | | 48 | | 43 | | 52 | | 59 | | 60 | |

c, Decrease in band intensities; d, increase in band intensities; a + b + c + d, varied band.

in Tables 4 and 5. In Tables 4 and 5, data shows that there is a direct relationship between Cd concentration and band changes, thus suggesting nonrandom interaction between DNA and Cd.

### Comparison of RAPD profiles, root growth and soluble protein content of roots in barley seedlings under cadmium stress

In Figure 4, the genomic template stability, a qualitative measure reflecting changes in RAPD patterns, was used to compare the modifications in RAPD profiles with reductions in root and shoot growth and soluble protein content of root tips in wheat seedlings. Following exposure to increasing Cd, root and shoot growth and soluble protein content of root tips in wheat seedlings decreased gradually. In contrast, the genomic template stability decreased after exposure to Cd too, but stabilized basically after 15 $mgl^{-1}$ Cd of exposure. The plateau effect was ascribed to multiple changes in RAPD profiles (for example appearance, disappearance of new bands), which tend to counterbalance each other. In other words, the disappeared band was compensated by the low frequency of newly appearing bands at 15 $mgl^{-1}$ Cd concentration for primers used in this experiment.

## DISCUSSION

In the present study, the application of RAPD marker analysis is reported for an assessment of cadmium genotoxicity in wheat. This technique has been used by many researchers for determination of genotoxic effect of heavy metal (Conte et al., 1998; Atienzar et al., 2002; Liu et al., 2005; Gupta and Sarin, 2009; Al-Qurainy, 2009; Liu et al., 2009; Cenkci et al., 2009; Taspinar et al., 2009; Shahrtash et al., 2010; Sameer and Qari 2010; Al-Qurainy et al., 2010). The concentration of Cd used in the present study induced some changes in DNA template which was detected by RAPD marker. Cadmium is neither an essential microelement for the growth of plants and animals, nor does it participate in the processes of cell metabolism. However, it is readily taken up probably through zinc transporter proteins (Suzuki, 2005; Fusconi et al., 2007), and it is toxic even at very low concentrations. Cadmium interacts with the root cell wall and, at the cellular level, it induces numerous physiological and metabolic disturbances (Liu et al., 2004; Suzuki, 2005; Fusconi et al., 2007). Cadmium enhances the production of reactive oxygen species that may damage major classes of macromolecules in plant cells, causing DNA strand breaks and increases membrane lipid peroxidation (Andrea, 1994; Fusconi et al., 2007). Metal ions can directly bind to DNA or indirectly through hydrogen bonding of the coordinating water molecules surrounding the metal ions. Metal binding to the bases usually disrupts base pair hydrogen bonding and destabilizes the double helix. The binding of metals to DNA and RNA also influences indirectly the

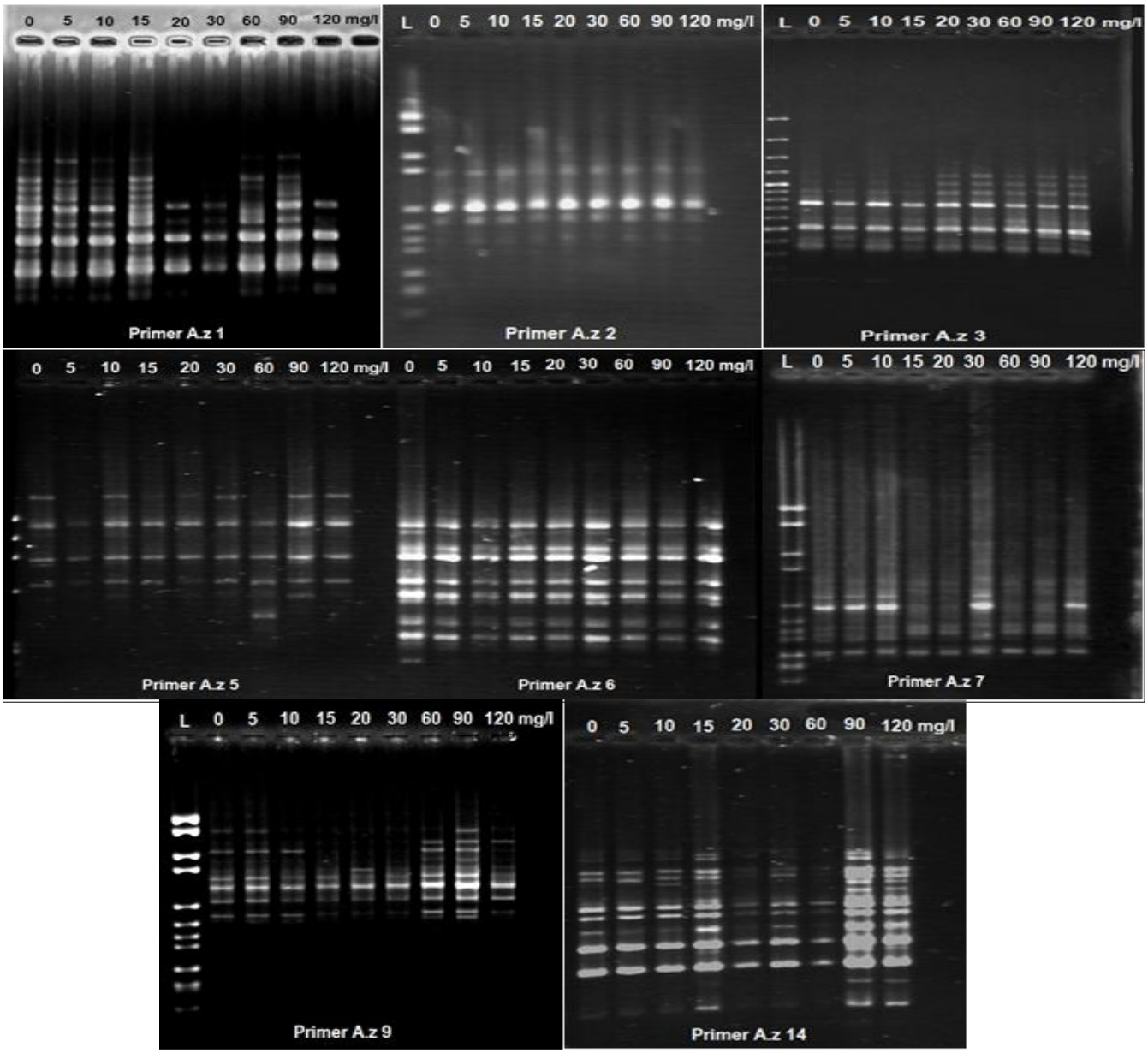


**Figure 3.** RAPD profiles of genomic DNA from root-tips of wheat seedlings exposed to varying Cd concentrations.

sugar conformation. As a result of this change in sugar puckering, the two helical conformations of A-DNA and B-DNA or RNA are characterized by the orientation of the bases with respect to the axis of the double helix (Valverde et al., 2001; Hossain and Huq, 2002; Anastassopoulou, 2003; Oliveira et al., 2008). This may be the reason why some metal ions influence DNA synthesis and the replication process. Like other metal ions, cadmium has direct interaction with DNA (Fuente et al., 2004; Oliveira et al., 2008). In this study, DNA damage was shown by RAPD profiles via disappearance or appearance and alteration in intensity (decrease or increase) of bands. Bands intensity in some primers under different concentration of cadmium showed some changes (increase or decrease). Changes observed in the DNA profiles such as modifications in band intensity and loss of bands may be due to the changes in oligonucleotide priming sites leading to genomic rearrangements, and less likely to point mutations or DNA damage in the primer binding sites which can block or reduce the efficiency of DNA polymerization in the PCR reaction (Atienzar et al., 2002; Liu et al., 2005; Gupta and Sarin, 2009; Gao et al., 2010). Appearance of new PCR products occurred because some oligonucleotide priming sites could become accessible to oligonucleotide primers after structural change or

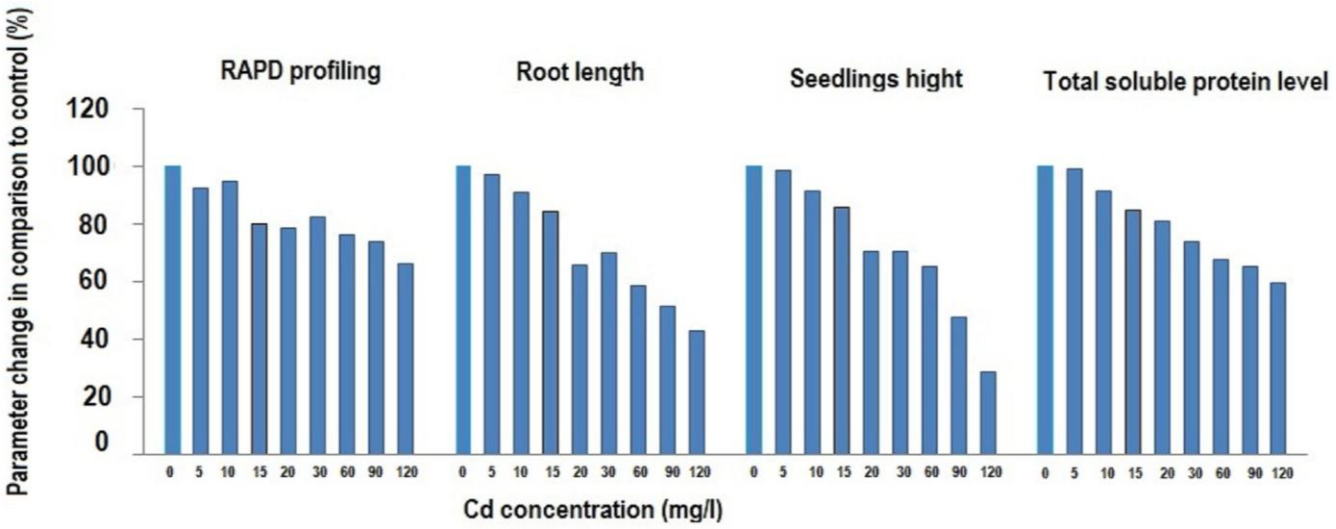


**Figure 4.** Comparison of root growth, plant height, total soluble protein level and genomic DNA template stability in root tips of wheat seedlings exposed to different Cd concentrations.

because some changes in DNA sequence have occurred due to mutations (resulting in new annealing events), and/or large deletions (bringing two pre-existing annealing sites closer), and/or homologous recombination (juxtaposing two sequences that match the sequence of the primer) (Atienzar et al., 1999; Liu et al., 2005). Trends in disappearing of RAPD bands in profiles in all primers were not similar. However, the number in high concentration was greater than in low concentration.

Disappearance of bands are likely to be due to changes in oligonucleotide priming sites, originating from rearrangements and less likely from point mutations and DNA damage in the primer binding sites (Liu et al., 2005; Enan, 2006; Liu et al., 2009). The disappearance of a normal RAPD product may be related to the events such as DNA damage (example, single and double strand breaks, modified bases, a basic sites, oxidized bases, bulky adducts, DNA–protein cross-links), point mutations and/or complex chromosomal rearrangements induced by genotoxins (Atienzar et al., 1999, 2000). The low concentration of Cd (5 and 10 $mgl^{-1}$) can also induce mutation and produce polymorphism in RAPD profiles. It was found that three informative 10-mer primers, Az.5, Az.12 and Az.13, may have great potential for detecting cadmium induced genomic alterations in low concentration. In Tables 4 and 5, data shows that there is a relative relationship between Cd concentration and RAPD profiles polymorphism, suggesting a nonrandom interaction between DNA and Cd.

Our finding on comparison between ‘untreated’ and ‘treated’ genomes show that RAPD analysis can be used for evaluation of toxicity on plants caused by environmental pollutants. On the basis of these considerations, we could suggest that RAPD technique is a powerful tool for measuring qualitative and quantitative genotoxic activities produced by cadmium. This method can be applied to a wide range of bioindicator organisms and may become a universal methodology to identify target genes for specific genotoxic agents .

In conclusion, the heavy metal such as Cd, can damage the seedlings of wheat and cause mutations even at low concentration. Since, wheat is immensely used as an important plant that provides human daily food and has an important role in human health; therefore, biomarkers are necessary for detection of heavy metals pollution in this species and its growing area to make human health free of hazardous materials. Thus, RAPD fingerprints appear to be stable in detection of genotoxic doses of those plants which are growing in heavy metal polluted soils.

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## REFERENCES

Al-Qurainy F (2009). Toxicity of heavy metals and their molecular detection on *Phaseolus vulgaris* (L.). Aust. J. Basic Appl. Sci. 3(3):3025-3035.

Al-Qurainy F, Abdulhafed A, Alameri SK (2010). RAPD profile for the assessment of genotoxicity on a medicinal plant; *Eruca sativa.* J. Med. Plant. Res. 4(7):579-586.

Anastassopoulou J (2003). Metal–DNA interactions. J. Mol. Struct. 651-653:19-26.

Andrea H (1994). Role of DNA repair inhibition in lead- and cadmium-induced genotoxicity. Environ. Health Perspect. 102:45-50.

Atienzar FA, Conradi M, Evenden AJ, Jha AN, Depledge MH (1999). Qualitative assessment of genotoxicity using random amplified polymorphic DNA: comparison of genomic template stability with key fitness parameters in *Daphnia magna* exposed to benzo[a]pyrene. Environ. Toxicol. Chem. 18:2275-2282.

Atienzar FA, Jha AN (2006). The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: A critical review. Mutat. Res. 613:76-102.

Atienzar FA, Paola V, Awadhesh NJ, Michael HD (2002). Evaluation of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage and mutations. Mutat. Res. 521:151-163.

Baryla A, Carrier p, Frank F, Coulomb C, Sahut C, Havaux M (2001). Leaf chlorosis oilseed rape plants *(Brassica napus)* grown on cadmium-polluted soil: Causes and consequences for phothosynthesis and growth. Planta 212(5-6): 696-709.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding analytical. Biochemistry 72:248-254.

Cenkci S, Mustafa Y, Ibrahim H, Cig E, Muhsin K, Ahmet B (2009). Toxic chemicals-induced genotoxicity detected by random amplified polymorphic DNA (RAPD) in bean (*Phaseolus vulgaris* L.) seedlings. Chemosphere 76:900-906.

Conte C, Mutti I, Puglisi P, Regina G, Maestr E, Marmiroli N (1998). DNA fingerprinting analysis by a PCR based method for monitoring the genotoxic effects of heavy metals pollution. Chemosphere 37:2739-2749.

Enan MR (2006). Application of random amplified polymorphic DNA (RAPD) to detect the genotoxic effect of heavy metals. Biotechnol. Appl. Biochem. 43(3):147-154.

Fuente LDM, Hernanz A, Navarro R (2004). IR and Raman study on the interactions of the 5-GMP and 5-CMP phosphate groups with Mg (II), Ca (II), Sr (II), Ba (II), Cr (III), Co (II), Cu (II), Zn (II), Cd (II), Al (III) and Ga (III). J. Biol. Inorg. Chem. 9(8):973-986.

Fusconi A, Gallo C, Wanda C (2007). Effects of cadmium on root apical meristems of *Pisum sativum* L. Cell viability, cell proliferation and microtubule pattern as suitable markers for assessment of stress pollution. Mutat. Res. 632:9-19.

Gao Y, Zhou P, Liang M, Yue-E Z, Wan-J S (2010). Assessment of effects of heavy metals combined pollution on soil enzyme activities and microbial community structure: modified ecological dose–response model and PCR-RAPD. Environ. Sci. 60:603-612.

Gupta M, Sarin BN ( 2009). Heavy metal induced DNA changes in aquatic macrophytes: Random amplified polymorphic DNA analysis and identification of sequence characterized amplified region marker. J. Environ. Sci. 21:686-690.

Hossain Z, Huq F (2002). Studies on the interaction between $Cd^{2+}$ ions and nucleobases and nucleotides. J. Inorg. Chem. 90:97-105.

Jin YH, Clark AB, Slebos, RJC, Al-Refai H, Taylor JA, Kunkel TA, Resnick MA, Gordenin DA (2003). Cadmium is a mutagen that acts by inhibiting mismatch repair. Nat. Genet. 34(3):326-329.

Joseph P (2009). Mechanisms of cadmium carcinogenesis. Toxicol. Appl. Pharmacol. 238:272-279.

Liu D, Jiang W, Gao X (2004). Effects of cadmium on root growth, cell division and nucleoli in root tips of garlic. Physiol. Plant. 47:79-83.

Liu W, Li PJ, Qi XM, Zhou QX, Zheng L, Sun TH, Yang YS (2005). DNA changes in barely (*Hordeum vulgare*) seedlings induced by cadmium pollution using RAPD. Chemosphere 61:158-167.

Liu W, Yang YS, Li PJ, Zhoua QX, Xiea LJ, Hana YP (2009). Risk assessment of cadmium-contaminated soil on plant DNA damage using RAPD and physiological indices. J. Hazard. Mater. 161:878-883.

Liu W, Yang YS, Zhou Q, Xie L, Li P, Sun T (2007). Impact assessment of cadmium contamination on rice (*Oryza sativa* L.) seedlings at molecular and population levels using multiple biomarkers. Chemosphere 67(6):1155-1163.

Oliveira SCB, Corduneanu O, Oliveira-B AM (2008). In situ evaluation of heavy metal DNA interactions using an electrochemical DNA biosensor. Bioelectrochemistry 72:53-58.

Sameer H, Qari M (2010). DNA-RAPD Fingerprinting and Cytogenetic Screening of Genotoxic and Antigenotoxic Effects of Aqueous Extracts of *Costus speciosus* (Koen.) JKAU Science 22(1):133-152.

Savva D (1996). DNA fingerprinting as a biomarker assay in ecotoxicology. Toxicol. Ecotoxicol. News 3:110-114.

Shafiq M, Iqbal MZ, Masol A (2008). Effect of lead and cadmium on germination and seedling growth of *Leucaena leucocephala*. J. Appl. Sci. Environ. Manag. 12(2):61-66.

Shahrtash M, Mohsenzadeh S, Mohabatkar H (2010). Cadmium-induced genotoxicity detected by the random amplification of polymorphism DNA in the maize seedling roots. Cell. Mol. Biol. 2(1):42-48.

Suzuki N (2005). Alleviation by calcium of cadmium-induced root growth inhibition in Arabidopsis seedlings. Plant Biotechnol. 22(1):19-25.

Taspinar MS, Guleray A, Nalan Y, Serap S, Ozkan A, Sedat B (2009). Evaluation of selenium effect on cadmium genotoxicity in *Vicia faba* using RAPD. J. Food. Agric. Environ. 7(3&4):857-860.

Theodorakis CW, Bickham JW, Lamb T, Medica PA, Lyne TB (2001). Integration of genotoxicity and population genetic analyses in kangaroo rats (*Dipodomys merriami*) exposed to radionuclide contamination at the Nevada Test Site, USA. Environ. Toxicol. Chem. 20(2):317-326.

Valverde M, Trejo C, Rojas E (2001). Is the capacity of lead acetate and cadmium chloride to induce genotoxic damage due to direct DNA–metal interaction. Mutagenesis 16(3):265-270.

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