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Genotoxic effects of cadmium on tolerant and sensitive wheat cultivars

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Abstract

In the present study, genotoxic properties of cadmium (Cd) in seedlings of wheat (*Triticum vulgare* L.) cultivars, chosen as cadmium tolerant (*Sönmez*-2001) and sensitive (*Quality*) seedlings, were evaluated by using randomly amplified polymorphic DNA (RAPD-PCR). After cadmium treatment, significant changes were observed on RAPD profiles of these cultivars. Genomic template stability (GTS) and cluster of similarity (UPGMA) were studied. GTS rates demonstrated that cv. *Quality*, which is a sensitive cultivar, was more affected. Furthermore, the distinguishing specific markers between tolerant and sensitive cultivars were determined. It can be concluded that DNA polymorphisms detected by RAPD-DNA, can be considered as a powerful biomarker assay for detection of the genotoxic effects of Cd stress.

Key words

Cadmium, Genomic Template Stability, Genotoxic effects, RAPD, Wheat

Introduction

Plants are affected by Cd which is non-essential and toxic heavy metal. Some plants are not affected during their growth when heavy metals of high amounts were exposed to these plants. Cd accumulation and translocation in plants varies based on their species and cultivars. Ability to absorb, accumulate and tolerate of Cd differs widely in crop species and cultivars (Miller *et al*., 2006). Metal ions can disrupt base pair hydrogen bonding and destabilize the double helix of DNA and RNA (Hossain and Huq, 2002; Anastassopoulou, 2003; Oliveira *et al*., 2008). Cd disturbs various biochemical and physiological processes; inhibits growth, damages different cellular components and cause mismatched repair of DNA in plants (Andrea, 1994; Cenkci *et al*., 2009, 2010). Some researchers have suggested that Cd ions interact with DNA through binding of Cd at G, A and T bases (Valverde *et al*., 2001; Anastassopoulou, 2003).

Randomly amplified polymorphic DNA (RAPD-PCR), which is a DNA-based molecular technique, can be used to detect genotoxicity (Theodorakis *et al*., 2006; Liu *et al*., 2009). A novel application of RAPD method as a biomarker assay is to detect DNA damage and mutational events such as rearrangement points of mutation, small insertion or deletions of DNA and ploidy changes in cells of bacteria, animals and plants (Conte *et al*., 1998; Atienzar and Jha, 2006; Liu *et al*., 2007; Aksakal *et al*., 2013). In view of the above, the present study was carried out to determine genotoxic effects of different level of Cd using molecular technique in two wheat (*Triticum vulgare* L.) cultivars.

Materials and Methods

Two wheat (*Triticum vulgare* L.) cultivars. Cd sensitive (*Quality*) and Cd tolerant (*Sönmez*-2001) were selected for study. These cultivars were chosen among 12 wheat cultivars. Seeds of wheat cultivars were obtained from Trakya Agricultural Research Institute and various firms. Seeds were surfaces were sterilized and then imbibed in distilled water for 24 hrs. The imbibed seeds were germinated at 25±1ºC in dark-controlled growth-room. Various concentrations of Cd 0.3 mM, 0.4 mM, 0.5 mM, 6 mM, 9 mM, 12 mM, and 16 mM as were added to each plate. These applications were repeated three times. Seeds treated with distilled water served as control.

DNA isolation and RAPD-PCR technique procedures : On day 10, seedlings of control and treated plants were ground in liquid

nitrogen. Total genomic DNA was extracted by using modified CTAB assay (Oard and Dronavalli, 1992). Purity and quality of DNA template was checked, both from 260/280 nm absorbance ratio and gel electrophoresis analysis. Reaction mixture (20 µl) of RAPD-PCR was prepared as follows: 2 µl of 10X PCR buffer; 0.7 µl of dNTPs (10 mM); 2.0 µl of magnesium chloride (25 mM); 1.44 µl of primer (100 pmol μl^{-1}); 0.2 µl of Taq polymerase (5 units μl^{-1}); 12.65 µl of distilled water and 1.0 µl (50 ng μl^{-1}) of sample DNA. Protocol of RAPD-PCR was prepared as follows: 3 min at 94°C, 40 cycles at 94°C for 30 sec, 36°C for 1 min, 72°C for 2 min and stored at 4 °C. All PCR examinations were carried out by Thermo (Px2 Thermal Cycler) and these amplifications were repeated twice. Total forty 10-mer oligonucleotide primers of 60-70% GC content were used; and among them, 15 primers were selected and used for future studies (Table 1). PCR products (20 µl) were mixed with 6x gel loading buffer (5 µl) and loaded on agarose (1.4 % w/v, with 4 µl Etbr/115 ml + 1xTBE buffer) gel electrophoresis in 1xTris-Borate-EDTA (TBE) buffer at 60V for 120 min. DNA bands were visualized and their photographs were taken under UV light (Spectroline-Bi-O Vision). DNA molecular size marker (50 bp marker; BioLabs) was used for each agarose gel.

RAPD profile and data analysis : The size and intensity of each amplification product were estimated using the Thermo myimage analysis software version 1.1. Only reproducible and clear amplification bands were scored for construction of data matrix. Polymorphism observed in RAPD-DNA profile included disappearance and/or appearance and variation in band intensity when compared with untreated control treatments was evaluated (Liu *et al.*, 2005; Cenkci *et al.*, 2009).

Genomic template stability (GTS) was calculated according to the formula of Cenkci *et al.* (2010). Genomic template stability (GST) was calculated as follows:

$$GST\% = (1- a/n) X 100$$

Table 1 : Sequences of 15 primers used in the study

Number of primers	Sequences of primers (5' →3')	%GC
Primer 1	TCT CCG CCC T	%70
Primer 2	AAG TCC GCT C	%60
Primer 3	AGT CAC TCC C	%60
Primer 4	TTC CCG GGT T	%60
Primer 5	ACC TTT GCG G	%60
Primer 6	ACG GCG TAT G	%60
Primer 7	AGG GCG TAA G	%60
Primer 8	AGG TGA CCG T	%60
Primer 9	ACC TCA GCT C	%60
Primer 10	CTG AGG TCT C	%60
Primer 11	CTG ATG CGT G	%70
Primer 12	CTG CGC TGG A	%70
Primer 13	CTG CTG GGA C	%70
Primer 14	CTG GCG AAC T	%70
Primer 15	TGG ACC GGT G	%70

Where, a: RAPD polymorphic profiles detected in each samples treated and n: the number of total bands in control. Polymorphism in RAPD profiles included disappearance of normal band and appearance of new band as compared to control. Mean was calculated for each experimental group exposed to different concentrations of Cd. In order to compare the sensitivity of each parameter, changes in these values were calculated, based on percentage of their respective control (set to 100 %).

Similarity of Qualitative Data (SIMQUAL) and SAHN module of Numerical Taxonomy and Multivariate Analysis System (NTSYSps) 2.1. program (Rohlf, 1994) were used for study similarity between cultivars and treated samples. Presence of each band was determined by binary matrix (1 for band presence and 0 for absence) for each sample. Numerical analysis was performed via hierarchical cluster analysis in order to compare the treated samples and control samples, in terms of banding pattern.

Results and Discussion

In the present study, the RAPD-DNA marker analysis was reported for assessing of Cd genotoxicity in sensitive and tolerant wheat cultivars. This technique has been widely used by researchers to determine the genotoxic effect of cadmium (Liu *et al*., 2005; Cenkci *et al*., 2009; Taspinar *et al*., 2009; Al-Qurainy *et al*., 2010). Table 2 and 3 illustrates summary of all polymorphic bands in RAPD-DNA (appearance, disappearance; increasing and decreasing band intensities). In control seedlings total number of bands was counted 161 in cv. *Quality* and 155 in cv. *Sönmez*-2001. The results revealed that RAPD-DNA profiles (appearance, disappearance) of treated and untreated groups showed difference in banding patterns. When control and treated wheat cultivars were compared, these differences observed in all RAPD-DNA profiles were clearly exhibited by appearance/ disappearance of some bands. Trends in RAPD-DNA profiles in all primers were not similar.

Maximum change in RAPD-DNA profiles was obtained in 9, 12 and 16 mM Cd concentrations in both the cultures, as compared to control. RAPD-DNA profiles of cv. *Quality* treated with different Cd concentrations were 1.8, 1.9, and 1.5 times greater as compared to cv. *Sönmez*-2001. Total, 127 RAPD-DNA bands disappeared as compared to control and 87 new bands appeared in Cd treated cv. *Quality*. However, in total 69 RAPD-DNA bands disappeared and 55 new bands appeared in Cd treated cv. *Sönmez*-2001. Briefly, number of disappeared and appeared bands in sensitive cv. *Quality* were higher as compared to tolerant cv. *Sönmez*-2001. However, when these cultivars were compared in terms of the band intensity, cv. *Sönmez*-2001 showed higher increase in band intensity at all Cd treatments as compared to cv. *Quality* .

Disappearance of bands may be related to DNA damage (e.g. single and double strand breaks, modified bases oxidized

Table 2 : RAPD profile alterations in number and intensities of bands as detected with 15 primer in Cd (mM) exposed wheat (cv. *Quality*) seedlings in comparison to the non-exposed control seedlings. (**a**) indicates appearance of new bands; (**b**) disappearance of normal bands; (**c**) decrease in band intensitie and (**d**) increase in band intensities. (**a+b**) denotes polymorphic bands and (**a+b+c+d**) varied band

Primers	C	Cadmium concentrations																									
		0.3				0.4				0.5				6				9				12				16			
		a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
1	11	2	0	7	3	2	1	0	7	4	1	0	10	4	1	2	8	8	2	3	6	0	5	2	2	4	2	1	8
2	11	0	2	6	2	0	1	7	2	1	1	5	4	1	2	6	1	2	1	4	4	0	8	3	0	3	2	4	5
3	11	0	0	2	8	4	0	2	7	2	0	4	7	2	0	2	7	4	0	6	4	0	2	5	3	2	3	5	5
4	8	1	0	2	5	1	0	6	0	2	0	0	6	2	1	4	2	2	1	3	3	4	2	3	2	1	2	2	2
5	9	1	0	1	3	1	0	1	4	2	0	1	4	2	0	2	3	4	1	1	4	2	4	2	3	4	0	1	2
6	9	0	1	2	6	1	1	3	5	2	2	5	2	2	1	3	5	2	2	3	5	2	4	3	2	2	2	3	2
7	12	2	1	3	5	3	2	5	3	3	2	5	3	1	2	7	3	3	3	5	3	1	4	6	1	3	4	5	3
8	8	1	0	1	6	2	0	1	6	3	0	5	3	4	0	3	4	4	1	2	4	3	1	2	5	2	0	1	5
9	10	0	1	4	4	0	0	2	6	0	1	8	1	0	0	5	5	0	0	2	5	1	4	4	1	0	0	0	9
10	15	0	4	3	7	0	3	6	6	0	3	5	5	1	6	1	6	2	7	3	4	0	7	2	6	1	6	4	5
11	9	0	0	2	7	1	0	1	8	1	1	7	0	0	0	2	7	1	0	4	5	1	4	2	3	1	0	1	8
12	12	1	2	6	4	1	2	5	5	1	2	4	6	3	4	4	3	3	4	2	6	3	5	2	2	4	5	3	3
13	10	0	1	5	1	0	1	2	6	0	1	5	3	0	0	5	3	0	0	3	5	1	5	4	0	1	2	3	5
14	18	0	4	5	8	0	1	5	12	0	8	9	1	0	3	6	7	0	4	4	8	0	9	5	3	0	5	9	4
15	8	2	1	6	0	0	0	7	0	0	0	5	2	1	0	1	7	4	0	1	6	2	4	4	0	0	0	8	0
Total bands	161	10	17	55	69	16	12	53	77	21	22	68	57	23	20	53	71	39	26	46	72	20	68	49	33	28	33	50	66
a+b		27				28				43				43				65				88				61			
a+b+c+d		151				158				168				167				183				170				177			

Table 3 : RAPD profile alterations in number and intensities of bands as detected with 15 primer in Cd exposed wheat (cv. *Sönmez*) seedlings in comparison to the non-exposed control seedlings. (**a**) indicates appearance of new bands; (**b**) disappearance of normal bands; (**c**) decrease in band intensities and (**d**) increase in band intensities. (**a+b**) denotes polymorphic bands and (**a+b+c+d**) varied band

Primers	C	Cadmium concentrations																									
		0.3				0.4				0.5				6				9				12				16			
		a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
1	10	0	1	8	1	3	0	0	9	3	1	1	5	2	0	1	8	4	0	0	5	0	1	5	3	0	0	2	7
2	9	0	0	0	1	1	0	0	9	2	0	1	8	0	1	1	7	1	1	1	7	1	3	5	0	2	2	0	7
3	11	4	0	2	3	1	0	1	7	4	0	1	6	1	0	2	9	0	0	0	11	0	0	3	7	0	0	3	7
4	9	1	1	4	3	1	1	3	4	1	2	0	3	0	1	0	7	1	0	1	4	1	2	2	3	2	2	1	5
5	10	2	0	2	7	2	0	3	7	2	0	0	8	0	1	0	9	2	0	0	10	1	2	0	8	0	1	0	8
6	8	4	0	0	7	0	0	0	7	4	0	0	8	0	5	1	1	4	0	0	8	1	2	0	6	1	2	1	5
7	12	2	1	9	2	2	1	3	8	1	0	2	8	0	2	2	8	2	2	2	8	2	5	0	5	2	3	1	6
8	9	2	0	2	6	3	0	4	5	3	0	6	2	2	1	4	4	2	1	5	3	0	2	0	6	1	0	7	1
9	10	0	1	2	4	0	0	0	8	2	0	0	10	2	0	0	10	2	0	0	10	0	2	2	5	0	3	0	7
10	15	1	1	5	8	0	2	2	9	1	2	2	9	1	2	3	9	2	3	0	12	1	6	1	8	1	6	4	5
11	10	0	0	3	5	0	0	2	6	0	0	1	9	1	0	2	8	1	0	1	9	1	0	0	10	1	0	1	8
12	11	0	0	4	7	0	0	0	11	0	0	0	10	0	1	0	10	0	0	0	10	2	1	1	9	2	1	0	10
13	9	1	0	6	3	1	0	5	4	2	0	6	2	1	0	6	2	0	0	4	5	1	1	1	5	0	1	2	5
14	12	4	0	6	6	4	0	4	8	4	0	4	8	3	0	2	8	4	0	2	9	1	2	2	8	1	2	1	7
15	10	0	0	0	8	2	0	2	7	3	0	0	10	1	2	0	8	1	2	0	8	2	4	0	6	2	4	1	4
Total bands	155	21	5	53	71	20	4	29	109	32	5	24	106	14	16	24	108	26	9	16	119	14	33	22	89	15	27	24	92
a+b		26				24				37				30				35				47				42			
a+b+c+d		150				162				167				162				170				158				158			

bases, and DNA-protein cross-links), point mutations and/or complex chromosomal rearrangements caused by genotoxic chemicals (Wolf *et al*., 2004; Atienzar and Jha, 2006). Cenkci *et al*. (2010) indicated that the total number of appeared and disappeared bands in RAPD profiles of lead-exposed *Brassica rapa* seedlings were almost same. Similar results were also observed in *Hordeum vulgare* and *Oryza sativa*, exposed to Cd (Liu *et al*., 2005, 2007; Cenkci *et al*., 2010).

Appearance of new bands occurred as few oligonucleotide priming were become accessible to oligonucleotide primers after structural change or since some changes in DNA sequence 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 matched the sequence of the primer) (Atienzar *et al*., 1999; Liu *et al*., 2005).

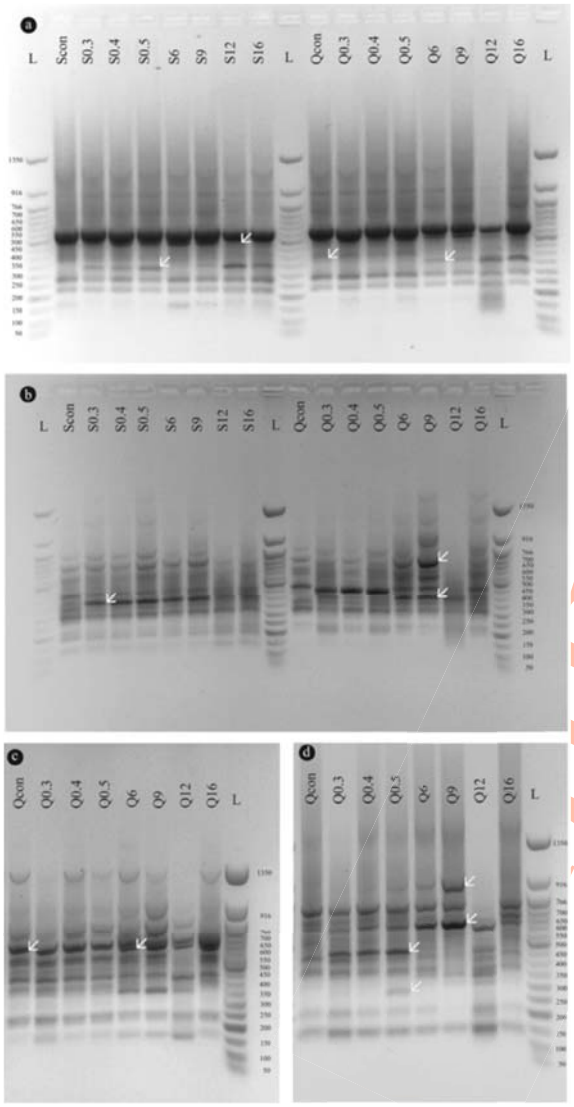

Fig. 1 : Randomly amplified polymorphic DNA profiles of Cd-exposed and nonexposed *wheat* seedlings with primers of (a) Primer-4, (b) Primer-5) c) Primer-6 and d) Primer-8 Arrows indicated new, disapperent, decrease and increase bands

Fig. 1 illustrates RAPD-DNA profiles of selected 4 primers (primer 4, 5, 6, and 8). Disappeared bands (440 kb) and increasing intensities of appeared bands (290 kb) in cv. *Sönmez*-2001 are shown in Fig. 1a. A decrease in band intensity (210 kb) was determined in cv. *Quality* at 6, 9, 12 and 16 mM Cd concentrations. Specific bands (350 kb) for cv. *Quality* were detected in control, 0.3 , 0.4 and 0.5 mM Cd concentrations. However, these specific bands disappeared in other Cd concentrations (Fig.1a). At 0.3 mM-9 mM Cd concentration, increase in band intensity was apparent in cv. *Sönmez*-2001 (345 bp) (Fig. 1 b). Increasing intensities of bands (360 bp, 690 bp) were significant at 6-9 mM Cd treatment in cv. *Quality* (Fig. 1 b). Appearance of new bands (690 bp) was significant at 6-16 mM Cd treatments in cv. *Quality* (Fig. 1c). New and intensive bands (450 bp) were determined at 0.3 , 0.4 and 0.5 mM Cd concentrations in cv. *Quality*. (Fig. 1d). Similarly, depending on the increase in Cd concentration (0.3 mM-9 mM), an increase in band intensity (620 kb) was noted (Fig. 1d). Furthermore, this study revealed that variability in band intensities (decrease or increase). Higher change in total band intensity was obtained in cv. *Sönmez*-2001. Maximum change in total band intensity was obtained at 0.4 mM Cd as compared to control samples in both wheat cultivars. (Tables 2 and 3).

Intensity of band may be the reason why some metal ions influenced DNA synthesis and the replication process. Like other metal ions, Cd has direct interaction with DNA (Fuente *et al*., 2004; Oliveira *et al*., 2008). Intensity of bands in some primers under different Cd concentrations showed some changes (increase or decrease). Changes observed in DNA profiles such as modifications in band intensity and loss of bands may be due to changes in oligonucleotide priming sites leading to genomic

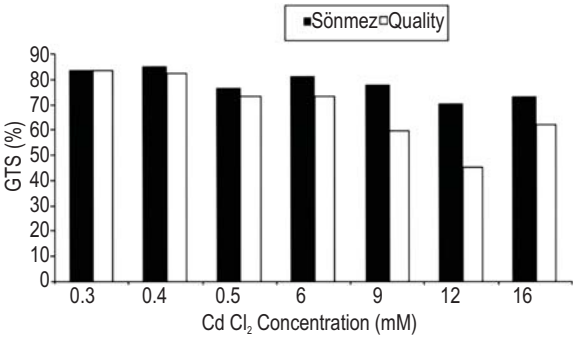

Fig. 2 : Changes in genomic template stability (GTS) values of all primers in wheat cultivars seedlings exposed to different Cd concentrations

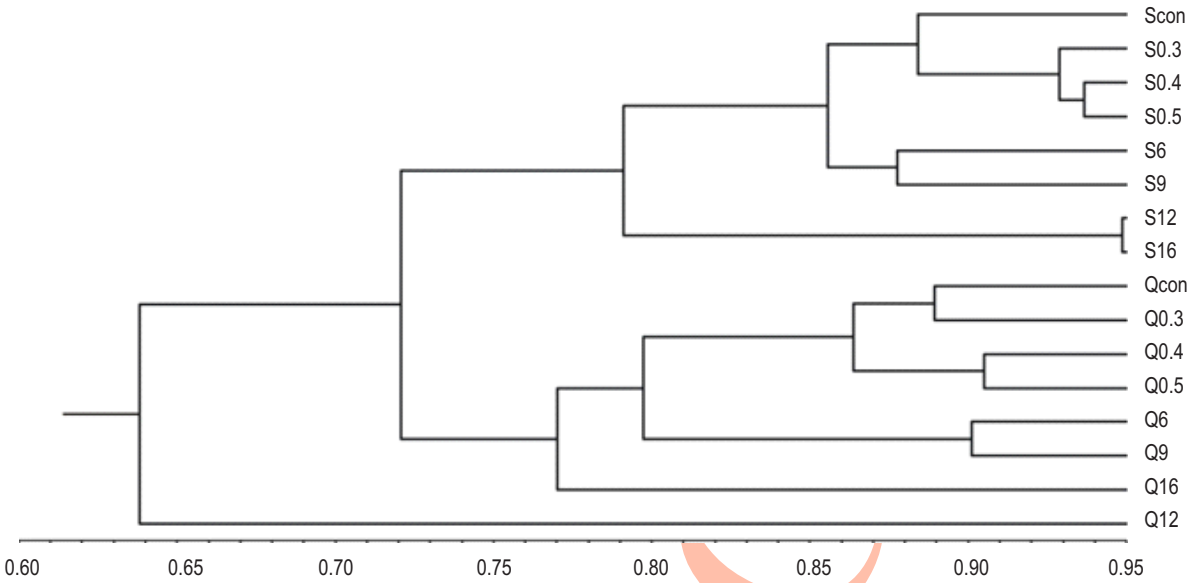

Fig. 3 : Cluster analysis (UPGMA) based on DNA polymorphism among the applicated and untreated (control) samples of Cd tolerant and sensitive wheat seedlings

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 PCR reaction (Atienzar *et al.*, 2002; Liu *et al.*, 2005; Gao *et al.*, 2010).

Changes in RAPD profiles due to genotoxic effect of heavy metals are reflected as values of genomic template stability (GTS), which is a qualitative method used to detect genotoxic effect of heavy metals and related to the level of DNA damage, efficiency of DNA repair and replication (Atienzar *et al.*, 1999, Cenkci *et al.*, 2010; Liu *et al.*, 2005). GTS (which is a quantitative measurement reflecting changes in RAPD patterns) was calculated for each 15 primers and presented in Fig. 2. It was observed that mean GTS values decreased obviously with an increase in Cd concentration especially in cv. *Quality*. When GTS rates determined by the RAPD profiles, were compared, it was observed that sensitive cultivar was more affected.

In *Quality* cultivar, GTS values were calculated as 83.23%, 82.61%, 73.29%, 73.2, 59.63, 45.3 and 62.11% for 0.3 , 0.4, 0.5, 6, 9, 12, 16 mM Cd concentration, respectively. At same concentration, cv. *Sönmez*-2001 GTS values obtained were obtained 83.23, 84.52, 76.13, 80.65, 77.42, 69.68 and 72.90%, respectively (Fig. 3). Higher GTS values obtained at 9, 12 and 16 mM Cd concentration might be an indication for introduction of an effective repair system or any other kind of cellular adaptation and/or defense system. Liu *et al.* (2005) stated that this situation was explained by the plateau effect ascribed to multiple changes in RAPD-DNA profiles which tend to counterbalance each other (Liu *et al.*, 2005).

The present results of Cd exposure in tolerant and sensitive wheat seedlings are compatible with results of previous studies conducted on other organisms. A similar effect on GTS has been reported due to Cd in *Oryza sativa* (Liu *et al.*, 2007) and copper in *Zea mays* (Xue-mei *et al.*, 2006). Liu *et al.* (2009) and Cenkci *et al.* (2010) reported that RAPD technique was more sensitive than classic genotoxic tests, since RAPD analysis was capable of detecting temporary DNA changes that may not finally manifest themselves as mutations.

A dendrogram constructed using RAPD-DNA profiles showed that cv. *Quality* and cv. *Sönmez*-2001 were separated in different clusters. It was observed that the applied samples of all cultivars were clustered in their internal Cd concentrations. This was more apparent especially in cv. *Sönmez*-2001. The reason for this was that there was specific band of cultivars. In the present study, different kinds and types of primers and specific markers were identified in control groups for distinguishing tolerant and sensitive cultivars. These markers for cv. *Quality* were as follows; thirty band of primer 1 (1014 bp), fifth band of primer 4 (345 bp), sixth band of primer 6 (454 bp), ninth band of primer 9 (314 bp), and sixth band of primer 10 (694 bp). These markers for cv. *Sönmez*-2001 were as follows; fifth band of primer 5 (337 bp), seventh band of primer 10 (425 bp), ninth band of primer 11 (224 bp) and forth band of primer 15 (695 bp). These markers would be widely applicable to study the effect of contaminants on population genetics and its adaptation to different stresses. DNA polymorphism was detected via RAPD analysis in terms of the presence or absence of bands in treatments by comparing with control. Thus, DNA polymorphism could be used as an

investigation tool for environmental toxicology and as a useful biomarker assay.

Results of the present study support the assertion that DNA polymorphisms detected by RAPD-DNA can be considered as a powerful biomarker assay for detection of genotoxic effects of environmental pollutants like Cd stress. These results also supported that RAPD method offers an applicable assay to determine the effects of heavy metals on DNA-DNA profiles.

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References

Aksakal O., F.A. Aygun, S. Sunar, S. Bozari and G. Agar: Assessment of genotoxic effects of 2,4- dichlorophenoxyacetic acid on maize by using RAPD analysis. *Ind. Crop Prod.*, **42**, 552-557 (2013).

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

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

Andrea, H.: Role of DNA repair inhibition in lead- and cadmium-induced genotoxicity. *Environ. Hlth. Persp.,* **102**, 45-50 (1994).

Atienzar, F.A., M. Conradi, A.J. Evenden and A.N. Jha: Depledge MH 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 (1999).

Atienzar F.A., V. Paola, N.J. Awadhesh and H.D. Michael: Evaluation of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage and mutations. *Mutat. Res.,* **521**, 151-163 (2002).

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

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

Cenkci, S., I.H. Cigerci, M. Yıldız, C. Özay, A. Bozdag, H. Terzi: Lead contamination reduces chlorophyll biosynthesis and genomic template stability in *Brassica rapa* L. *Environ. Exp. Bot.,* **67**, 467-473 (2010).

Conte, C., I. Mutti, P. Puglisi and A. Ferrarini: DNA fingerprinting analysis by a PCR based method for monitoring the genotoxic effects of heavy metals pollution. *Chemosphere*, **37**, 2739-2749 (1998).

Fuente, L.D.M., A. Hernanz and R.Navarro: 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**, 973-986 (2004).

Gao, Y., P. Zhou, M. Liang, E.Z. Yue and J.S. Wan: 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 (2010).

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

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

Liu, W, Y.S. Yang, P.J. Li, Q.X. Zhoua, L.J. Xiea and Y.P. Hana: Risk assessment of cadmium-contaminated soil on plant DNA damage using RAPD and physiological indices. *J. Hazard. Mater.,* **161**, 878-883 (2009).

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

Miller, J.F., C.E. Green, Y.M. Li and R.L. Chaney: Registration of three low cadmium (HA 448, HA 449, and RHA 450) confection sunflower genetic stocks. *Crop. Sci.,* **46**, 489-90 (2006).

Oard, J.H. and S. Dronavalli: Rapid isolation of rice and maize DNA for analysis by random-primer PCR. *Plant Mol. Biol. Rep.,* **10**, 236-241 (1992).

Oliveira, S.C.B., O. Corduneanu and A.M. Oliveira-B: In situ evaluation of heavy metal DNA interactions using an electrochemical DNA biosensor. *Bioelectrochemistry*, **72**, 53-58 (2008).

Rohlf, F.J.: Numerical Taxonomy and Multivariate Analysis System. Version 1.70. Exeter Software, Setauker, N.Y. (1994).

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

Theodorakis, C.W., K.L. Lee, S.M. Adams and C.B. Law: Evidence of altered gene flow, mutation rate, and genetic diversity in redbreast sunfish from a pulp-mill-contaminated river. *Environ. Sci. Technol.*, **40**, 377-386 (2006).

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

Wolf, H.D., R. Blust and T. Backeljau: The use of RAPD in ecotoxicology. *Mutat. Res.*, **566**, 249-262 (2004).

Xue-mei, Q., L. Pei-jun, L. Wan and X. Li-jing: Multiple biomarkers response in maize (*Zea mays* L.) during exposure to copper. *J. Environ. Sci.*, **18**, 1182-118 (2006).