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DETERMINATION OF HEAVY METAL-INDUCED DNA DAMAGE IN *PISUM SATIVUM* L. AT THE MOLECULAR AND POPULATION LEVEL

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ABSTRACT

In the current study, the effects of heavy metals on pea (*Pisum sativum* L.), which were exposed to 150 mg L^{-1} and 300 mg L^{-1} Cd, Pb and Cu treatments for 12 days, were investigated by using population parameters and Random Amplified Polymorphic DNA (RAPD) assay in the roots and leaves. In RAPD assay, 17 RAPD primers generated polymorphic band profiles, and a total of 110 bands were produced in the leaves and roots of the control seedlings. Changes were detected in the RAPD patterns of the roots and leaves of the pea plants that subjected to heavy metal treatment in terms of band intensities, appearance of new bands and disappearance of bands. The changes in the RAPD patterns were found to be more in roots when compared leaves. The results of germination, shoot and primary root length showed a correlation with the genomic template stability (GTS) values. Determination of the DNA damage by using the RAPD assay together with different biomarkers may be a practical eco-genotoxicological tool in the biomonitoring of chemical contaminants.

Keywords: Pea (*Pisum sativum* L.), heavy metal, genotoxicity, growth parameters.

INTRODUCTION

In the last thirty years, researchers have become aware the importance of environmental stress on human health and ecosystems considerably. These concerns have been strengthened by the global climate change, unplanned release of radioactive gases and industrial chemicals and the serious increase in reports about oil spills (Bickham, 2000). In addition, growing human population and its agricultural, industrial and defense-related activities have led to the formation and rapid dispersion of chemical contaminants (Vitousek *et al.,* 1997). Among these contaminants, heavy metals with genotoxic effects have increasing significance due to toxicity on environmental and ecological reasons (Vitousek *et al,.* 1997; Nagajyoti *et al.,* 2010).They are not chemically degraded in soil and cause cytotoxic effect when they accumulate more than the cell's need (Conte *et al.,* 1998).

Cadmium (Cd), mercury (Hg), lead (Pb), chromium (Cr) and copper (Cu) are prevalent contaminants especially in environment as a result of human activity (Nagajyoti *et al.,* 2010). At the beginning of 1990s, estimated annual release of cadmium reached 22000 tones worldwide and a vast scale of this amount was determined to be found in soil (Fusconi *et al.,* 2007; Liu *et al.,* 2007). Lead shows high durability in environmental conditions and because of the use in many industrial activities from ancient ages, it is a major environmental pollutant around the world (Garcia-Leston *et al.,* 2010). Copper, an essential element for plants, causes oxidative stress when found in high concentrations in soil (Muccifora *et al.,* 2007).

By DNA-based techniques, genotoxicity can be evaluated inclusively on the DNA of exposed species and moreover, information can be achieved about a large number of loci along the genome (Noel and Rath, 2006). Among these techniques, Random Amplified Polymorphic DNA (RAPD), DNA fingerprints of individuals subjected to and not subjected to genotoxic agents are compared and genotoxicity can be determined by the differences among RAPD profiles (Atienzar and Jha, 2006; Enan, 2006).

As a good bioindicator, higher plants present genetic systems practical for screening and monitoring of environmental contaminants (Conte *et al.,* 1998). Bioindicators make possible to determine the subtle forms of pollution in areas in which the measurement of pollutants is difficult (Al-Qurainy *et al.,* 2010). The objective of the present study was to determine DNA alterations induced by heavy metals (Cd, Cu and Pb) in leaves and roots of pea seedlings via RAPD assay and to compare GTS values with population parameters such as germination, shoot and primary root length.

MATERIALS AND METHODS

Plant material and growth conditions: Pea, *Pisum sativum* L., seeds were surface-sterilized with 10% sodium hypochloride (5% active chloride) for 10 min, then dipped in 96% ethanol for 10 min and rinsed in distilled water five times (Fusconi *et al.,* 2006). Pea seeds

were germinated between three layers of sterile saturated filter paper at dark, 23-25ºC temperature in sterile magenta vessel. Three days after sowing, uniform germinated seeds were transferred to growth chamber (22-25ºC day/night, 16/8h photoperiod, 200 µmol m^{-2} s^{-1}, 65-70% relative humidity) and seedlings exposed to 150 and 300 mg L^{-1} cadmium cloride ($CdCl_2$), lead nitrate [$Pb(NO_3)_2$], copper sulfate ($CuSO_4.5H_2O$) or distilled water (control) for 12 days. Concentrations were chosen on the basis of previous study (Enan, 2006). The pea seedlings were treated with heavy solution for a total of 5 times as 5 ml at three day intervals and samples collected 24 hours after the last treatment. Four seeds were germinated in each magenta vessel and 20 magenta vessels were used for each treatment group. At the end of the 12 days, germination of seeds (%) and shoot and primary root length (cm) of 10 pea seedlings were recorded and statistical analyses were performed.

Genomic DNA extraction and Random Amplified Polymorphic DNA (RAPD) assay: Genomic DNA was isolated from leaves and roots by CTAB protocol with minor modifications (Surgun *et al.,* 2012). About 0.4 – 0.5 g of frozen leaf and root samples were collected from 10 pea seedlings and powdered in liquid nitrogen. The concentrations and purities of the DNA samples that were extraced from the roots and leaves of the pea seedlings was determined spectrophotometrically varied between 180 and 200 mg $^{-1}$ FW and 1.6 and 2.1, respectively. The quality and integrity of all the DNA that were isolated were checked in agarose gel (1 %) and single band was observed (Fig.1). All DNA samples were diluted with distilled water as the last concentration is 5 ng μL^{-1}. In order to quantify DNA changes, RAPD assay was used. RAPD-PCR was done in a volume of 15 µl, containing 1.5 µl of 10X Taq buffer with KCl, 0.25 µL Taq DNA polymerase, 1 µL dNTP (10 mM), 1 µl primer (10 µM), 2.4 µl $MgCI_2$ (25 mM), 25 ng genomic DNA and 3.85 µL ddH_2O. Initially 26 random RAPD primers were performed and among them 22 primer amplified clean and repeatable bands for RAPD assay. In Table 1, sequences of 22 primers utilized in this study were shown. Amplifications were carried out in thermocycler (BioRad T100, ABD) as follows: 1 minute at 94 ºC (initial denaturating step), 45 cycles of 1 min at 94ºC (denaturation), 1 min at 36 ºC (annealing), 2 min at 72 ºC (extension) and 10 min at 72ºC (final extension) following the last cycle. A negative control was run with each sample set. The RAPD amplicons were analysed in 2% agarose gel including safe DNA gel stain (Invitrogen, USA) by electrophoresis using 1X TAE (MULTIcell, Canada). Photos of gels were captured under UV light by using Fusion FX7 imaging system (Vilber Lourmat, Germany). To verify the reproducible RAPD patterns, all primers were repeated at least 3 times. Magnitude of amplification products were automatically determined by the Fusion-CAPT-Software 16.07. A 1.0-kilobase DNA Ladder (Thermo Scientific, Germany) was run as a marker in each gel. Ladder lanes on gels were visualized from top to down as 10000, 8000, 6000, 5000, 4000, 3500, 3000, 2500, 2000, 1500, 1000, 750, 500 and 250 bp.

Estimation of genetic template stability (GTS): Genomic Template Stability (GTS) value was calculated with this formula: GTS (%) = (1- a / n) x 100 where 'a' is the number of polymorphic bands determined in each treated sample and 'n' is the total number of control bands (Atienzar *et al.,*1999). Alterations in these values were calculated as a percentage of their control (set to 100%) in order to compare the sensitivity of parameter (GTS, germination, primary root length, shoot length).

Statistical analysis: The parametric assumptions (data normality and homoscedasticity) were tested using Shapiro-Wilk's and Bartlett's tests for each data set. If needed, several data transformations (e.g. arcsine and logarithmic) were done to obtain normal distribution. In order to compare the difference between heavy metal treatments and control, germination percentage, shoot length and primary root length data were analysed by one-way analysis of variance (ANOVA) followed by Dunnett's test. In these analyses control and heavy metal treatments were considered as categorical factor, while germination and seedling growth data were taken as dependent variables. In all analyses, the significance level was set at 0.05.

RESULTS

The effect of Cd, Pb and Cu treatments, which were performed at various concentrations, on germination and shoot and primary root length in the pea seedlings are given in Table 2. All heavy metal treatments reduced the seed germination, shoot and primary root lengths at a statistically significant level compared with their respective control (Table 2). At the end of the 12 days, 150 mg L^{-1} Cd, Pb and Cu treatments caused that the germination rates decreased at a rate of 23.6%, 26.8% and 30.1%, respectively; and the 300 mg L^{-1} Cd, Pb and Cu treatments caused that the germination rates were decreased at a rate of 46.2%, 49.4% and 55.9%, respectively when compared with the control. The length of the shoot and primary roots in the pea seedlings decreased depending on the increase in the concentration of heavy metal (Table 2).

RAPD assay was performed in the DNA samples extracted for the purpose of determining possible DNA damage in the tissues of the leaves and roots caused by heavy metals used in the study. 26-10 mer primer was used for the scanning of the changes in the pea genomes and the 22 primers (84.61 %) gave clean and repeatable bands.While there were differences in the RAPD patterns

of the heavy metal-exposed seedlings and control seedlings in 17 primers (77.27 %) out of the 22 primers, no differences were detected in 5 primers (22.73 %) (OPB-04, OPB-08, OPM-15, OPM-16, and OPM-19). Seventeen primers produced 110 bands in the control (leaf and root samples). When the RAPD patterns of the control seedlings were compared with the leaves and roots of the heavy metal-exposed pea seedlings, there were changes in the number (appearance of new bands or/and disappearance of normal bands), size and increase and decrease of band intensity of the DNA fragments.The differences determined in the RAPD profile of the root and leaf samples of treated seedlings are summarized in Table 3 and 4, respectively. The RAPD profiles of 2 selected primers in the root and leaf samples are shown in Fig. 2.

Among the primers that were used in the study, while the OPB-18 primer generated polymorphic bands (8 bands) at the highest number between 600 bp and 2000 bp in the roots, the OPB-15 and OPM-13 primers produced only 2 polymorphic bands that changed between 580 bp and 2500 bp (Table 3). In the leaves of the pea seedlings, the OPA-01 primer gave 9 polymorphic bands that changed between 310 bp and 2200 bp, OPM-10 and OPM-12 primers produced 3 polymorphic bands that changed between 495 bp and 2655 bp (Table 4).

The number of the total new appeared and disappeared bands that were observed as a result of the heavy metal treatments at higher concentration (300 mg L^{-1} Cd, Pb and Cu) was more than the number of the bands that were observed in the treatments in low concentration (150 mg L^{-1} Cd, Pb and Cu) in the roots (Table 5). Similarly, while the number of the total new appeared and disappeared bands was 23 and 93, respectively at higher concentration as a result of the heavy metal treatments at higher concentration, these were 13 band and 73 band at lower concentration in the leaves (Table 5).

Decreases and increases in band intensities were determined in the RAPD patterns of the roots and leaves of the pea seedlings that were exposed to heavy metal treatments at different concentrations (Table 5). The total band intensity changes (increased and decreased band intensity) that occurred as a result of 150 mg L^{-1} Cd, 300 mg L^{-1} Cd, 150 mg L^{-1} Pb, 300 mg L^{-1} Pb, 150 mg L^{-1} Cu and 300 mg L^{-1} Cu treatments in the roots were 19, 25, 24, 26, 24, and 27, respectively; and 14, 17, 11, 21, 17, and 21, respectively in the leaves (Table 5).

The average polymorphism values for different heavy metal treatment groups are given in Table 6. Polymorphism rates increased with increasing heavy metal concentrations, while genomic template stability (GTS) decreased in roots and leaves of pea seedlings. The average GTS values in average in the roots were determined in 150 mg L^{-1} Cd and 300 mg L^{-1} Cd treatment as 73.48%, 51.66%, in 150 mg L^{-1} Pb and 300 mg L^{-1} Pb treatment as 50.29%, 44.35%; in 150 mg L^{-1} Cu and 300 mg L^{-1} Cu treatment as 56.24% and 43.44% (Table 6). The average GTS values in the leaves were found to be 74.14%, 66.74%, 72.34%, 61.91%, 70.82%, and 59.13% for the 150 mg L^{-1} Cd, 300 mg L^{-1} Cd, 150 mg L^{-1} Pb, 300 mg L^{-1} Pb, 150 mg L^{-1} Cu and 300 mg L^{-1} Cu treatments in respective order (Table 6). Following the 12-day application, GTS values of the roots and leaves showed similar trend with germination percentage and growth parameters, and decreased depending on the increasing heavy metal concentration (Fig. 3).

Table 1. Sequences of 22 primers used in the study

Primer number	Primer name	Primer sequence (5'→3')	Primer number	Primer name	Primer sequence (5'→3')
1	OPA-01	CAGGCCCTTC	12	OPM-01	GTTGGTGGCT
2	OPB-04	GGACTGGAGT	13	OPM-02	ACAACGCCTC
3	OPB-05	TGCGCCCTTC	14	OPM-04	GGCGGTTGTC
4	OPB-06	TGCTCTGCCC	15	OPM-05	GGGAACGTGT
5	OPB-07	GGTGACGCAG	16	OPM-06	CTG GGCAACT
6	OPB-08	GTCCACACGG	17	OPM-10	TCTGGCGCAC
7	OPB-10	CTGCTGGGAC	18	OPM-12	GGGACGTTGG
8	OPB-15	GGAGGGTGTT	19	OPM-13	GGTGGTCAAG
9	OPB-17	AGGGAACGAG	20	OPM-15	GACCTACCAC
10	OPB-18	CCACAGCAGT	21	OPM-16	GTAACCAGCC
11	OPH-18	GAATCGGCCA	22	OPM-19	CCTTCAGGCA

Table 2. Effects of different concentrations of Cd, Pb and Cu on germination percentage, shoot length and primary root length of pea. Values are given as mean ± SE (n = 10)

Parameters	Control	Treatments					
		Cd		Pb		Cu	
		150 mg L^{-1}	300 mg L^{-1}	150 mg L^{-1}	300 mg L^{-1}	150 mg L^{-1}	300 mg L^{-1}
Germination (%)	93.0 ± 4	71.0 ± 6.0*	50.0 ± 5.0**	68.0 ± 5.0*	47.0 ± 4.0**	65.0 ± 4.0**	41.0 ± 5.0**
Shoot length (cm)	4.5 ± 0.3	3.2 ± 0.2**	2.4 ± 0.1**	3.0 ± 0.1**	2.2 ± 0.1**	3.2 ± 0.2**	2.3 ± 0.1**
Primary root length (cm)	8.6 ± 0.2	6.0 ± 0.3**	5.1 ± 0.2**	5.8 ± 0.3**	4.3 ± 0.2**	4.8 ± 0.2**	2.7 ± 0.2**

* $P < 0.05$; ** $P < 0.001$ according to Dunnett's test.

Table 3. The number of bands in control (C) and molecular sizes (base pair, bp) of disappearance (−) and/or appearance (+) of DNA bands for all used primers in the roots of heavy metal treated pea seedlings in comparison to the control

Primers	C		Cd (mg L^{-1})		Pb (mg L^{-1})		Cu (mg L^{-1})	
			150	300	150	300	150	300
OPA-01	10	−	1390; 310	1970; 1390	1970; 880	1970; 880; 310	1970; 880	1970; 880
		+	1080	1080	1080	1080	1080	1080
OPB-05	5	−	800; 530	0	0	1000; 530	0	0
		+	1850; 1100	1850; 1100	1850; 1200; 1100	1850; 1200; 1100	1850; 1200; 1100	1850; 1200; 1100
OPB-06	8	−	0	2655; 1960; 1300	2655;1960; 1300	2655; 1960	2655; 1960; 1300	2655; 1960; 1300
		+	0	0	0	0	0	0
OPB-07	5	−	0	440	3700;440	3700	2300;440	2300;440
		+	0	1750; 1050; 560	1750; 1050; 560	1750; 1050; 560	3330; 1750; 1050; 560	1750; 1050; 560
OPB-10	5	−	1070; 850	2200; 1070	2200;1070	2200; 850	2200; 1520; 1070	2200; 1520; 1070
		+	1780	1780	1780	1780;1270	1780	1780; 1270; 550
OPB-15	3	−	0	580	580	0	0	580
		+	0	1025	1025	1025	0	0
OPB-17	8	−	900; 658	2820; 1650; 1000	2820; 1000; 520	2820; 1650; 1000	0	1650; 1000
		+	0	770	770	770	770	770
OPB-18	6	−	1570	2000; 1780; 1570;950	2000; 1780; 1570; 950	2000; 1780; 1570; 950	2000; 1780; 1570; 950	2000; 1780; 1570; 950; 600
		+	1100; 810	1420	1420	1420	1420	1420
OPH-18	6	−	0	0	0	1500	1500	1500
		+	0	0	780	860; 780	860; 780	860; 780
OPM-01	7	−	0	1825; 1300; 1050	1825; 1300; 1050	2010; 1825; 1300; 1050	1825; 1050	1825; 1050
		+	0	0	0	0	580	0
OPM-02	9	−	1190	780; 690; 410	780; 690; 410	2300; 1190; 780; 690; 410	690; 410	2300; 780; 690; 410
		+	0	0	0	2000	2000	2000; 385
OPM-04	9	−	1130; 830	1130; 830; 680	1850	3100; 2150; 1850; 1290	2150; 1850; 1290	3100; 2150; 1850; 1290
		+	0	1420	0	0	0	0

OPM-05	8	−	2210; 1920; 1300; 1000	2210; 1440; 1300	2210; 1440; 1300	1440; 1300	2210; 1440	2210; 1440; 1300
		+	900	900	900	900	900	900
OPM-06	5	−	2400; 610	0	0	2400	0	0
		+	0	1030; 540	1030; 540	1030; 540	0	1030; 540
OPM-10	5	−	1438	1438	1438; 610	2155; 1438	1438	2155; 1438; 610
		+	0	930	930	930	930	930
OPM-12	4	−	1770; 495	1770; 1050	1770; 1050	1770	1770; 1050	1770; 1050
		+	0	836	836	836	320	320
OPM-13	7	−	0	2500; 1900	2500; 1900	2500; 1900	2500; 1900	2500; 1900
		+	0	0	0	0	0	0

Table 4. The number of bands in control (C) and molecular sizes (base pair, bp) of disappearance (−) and/or appearance (+) of DNA bands for all used primers in the leaves of heavy metal treated pea seedlings in comparison to the control

Primers	C		Cd (mg L^{-1})		Pb (mg L^{-1})		Cu (mg L^{-1})	
			150	**300**	**150**	**300**	**150**	**300**
OPA-01	10	−	880	1970; 1630; 381	1390	381	1190	1190; 310
		+	1500	2200	0	0	0	0
OPB-05	5	−	1550; 530	1550; 530	800; 530	1000; 800; 530	530	530
		+	0	3700	0	0	3700; 900	3700; 900
OPB-06	8	−	1960; 1500	1960; 1500	1960; 1500	1500	2655; 1960	2655; 1960
		+	0	0	0	0	0	0
OPB-07	5	−	3700	3700	0	3700	3700	0
		+	835	0	0	835;750	0	2900
OPB-10	5	−	1520; 1070; 850	2200; 850	2200; 1520; 1070; 850	2200; 1520; 1070; 850	850	1070; 850
		+	0	1780	1780	1780	0	1780
OPB-15	3	−	1220	1220	0	0	0	0
		+	1025	1025	0	870	0	870
OPB-17	8	−	0	2820; 1000; 658; 520	520	900; 658; 520	2820	2820; 1000; 658; 520
		+	770	0	0	0	0	0
OPB-18	6	−	950	1780; 950	950	2000; 1570; 950	2000; 1780; 950	2000; 1780; 950
		+	0	810	0	810	0	0
OPH-18	6	−	0	0	450	450	1080	1080; 450
		+	780	860; 780	780	780	0	0
OPM-01	7	−	1050	1050	2040; 1050	0	1050	1050
		+	0	0	580	1180	580	1180
OPM-02	9	−	0	0	690; 410	2300; 1190; 780; 690; 410	690; 410	690; 410
		+	0	0	0	0	0	0
OPM-04	9	−	830	830	830	830	830	1850; 830
		+	0	0	1420	1420	1420	1420
OPM-05	8	−	2210; 1920; 800	2210; 1920	1190	1190; 800	2210; 1920; 1190	2210; 1920; 1190
		+	0	0	0	0	0	0
OPM-06	5	−	1945; 1300	1945; 1300	1945; 1300	1945; 1300	2400; 1945; 1300	2400; 1945; 1300
		+	0	0	0	0	0	0

OPM-10	5	−	0	2155	0	0	2155; 1438	2155; 1438
		+	0	0	0	930	0	0
OPM-12	4	−	1770	1050;495	1770; 1050	0	0	1770; 1050
		+	0	0	0	0	0	0
OPM-13	7	−	1320	0	2500; 1320; 430	2500; 1320; 650; 430	2500; 1320; 845; 650; 430	2500; 1320; 845; 650; 430
		+	0	0	0	0	0	0

Table 5. RAPD profile changes in total number of polymorphic and intensities of bands (*a*, *b*, *c* and *d*) as detected with all used primers in roots and leaves of pea seedlings after 12 days of heavy metal treatments in comparison to the control

Heavy metal treatments (mg L^{-1})	Root				Leaf			
	a	***b***	***c***	***d***	***a***	***b***	***c***	***d***
Control	-	-	-	-	-	-	-	-
Cd150	7	21	8	11	5	20	9	5
Cd300	17	33	15	10	7	26	12	5
Pb150	17	33	13	11	4	25	5	6
Pb300	20	39	19	7	9	31	11	10
Cu150	18	29	13	11	4	28	10	7
Cu300	21	39	13	14	7	36	11	10

**a* denotes appearance of new bands, *b* disappearance of normal bands, *c* decrease in band intensities, *d* increase in band intensities.

Table 6. The average polymorphism (%) rates and genomic template stability (%) values in the roots and leaves of pea seedlings exposed to different heavy metals for 12 days

Heavy metal treatments (mg L^{-1})	Root		Leaf	
	Polymorphism (%)	GTS (%)	Polymorphism (%)	GTS (%)
Control	0	100	0	100
Cd150	25.45	73.48	22.73	74.14
Cd300	45.45	51.66	30.00	66.74
Pb150	45.45	50.29	26.36	72.34
Pb300	53.64	44.35	36.36	61.91
Cu150	42.73	56.24	29.09	70.82
Cu300	54.55	43.44	39.09	59.13

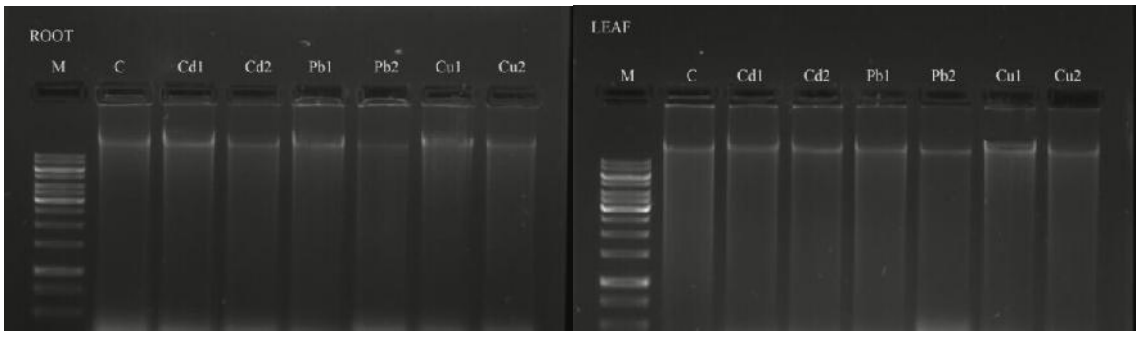

Figure 1. The quality of DNA extracted from root and leaf samples of pea seedlings subjected to heavy metal at different concentrations for 12-days (C: Control, Cd1: 150 mg L^{-1} Cd, Cd2: 300 mg L^{-1} Cd, Pb1: 150 mg L^{-1} Pb, Pb2: 300 mg L^{-1} Pb, Cu1: 150 mg L^{-1} Cu, Cu2: 300 mg L^{-1} Cu). M: DNA molecular size marker (1.0-kb).

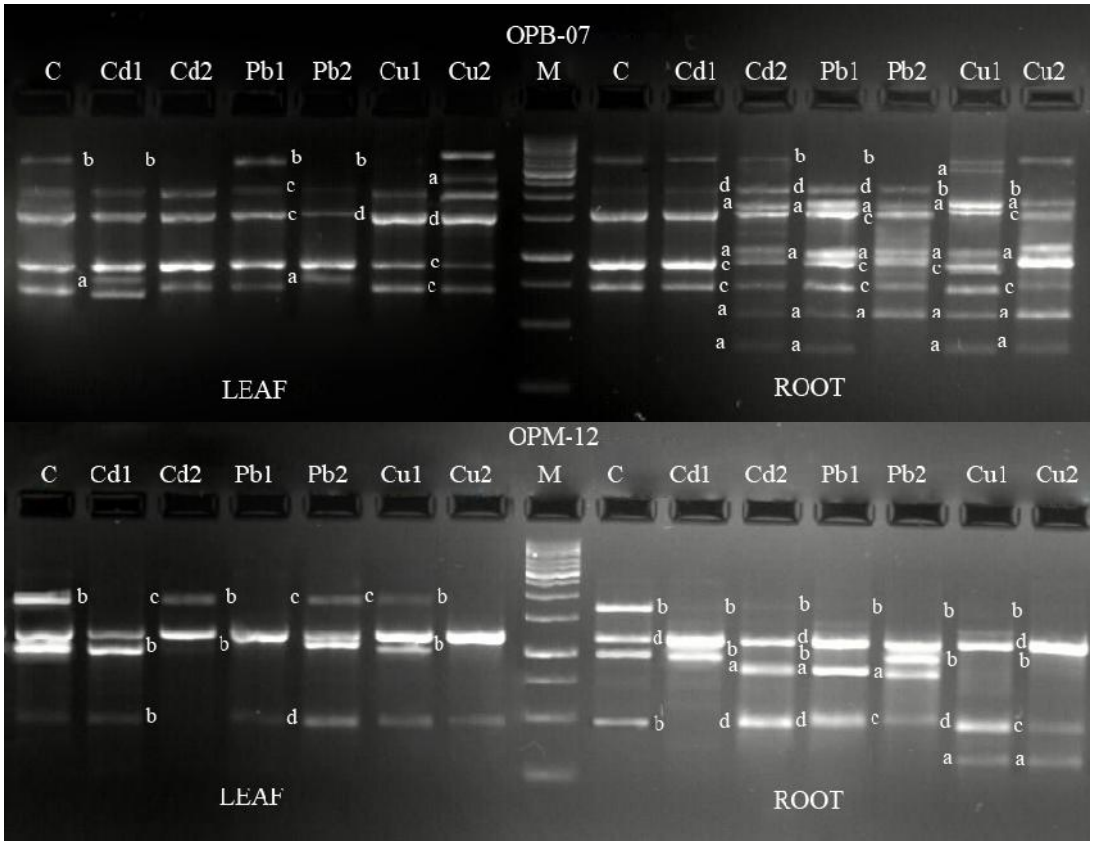

Figure 2. RAPD profiles of OPB-07 and OPM-12 primers of genomic DNA from leaves and roots of heavy metal treated pea seedlings (C: Control, Cd1: 150 mg L^{-1} Cd, Cd2: 300 mg L^{-1} Cd, Pb1: 150 mg L^{-1} Pb, Pb2: 300 mg L^{-1} Pb, Cu1: 150 mg L^{-1} Cu, Cu2: 300 mg L^{-1} Cu). M: DNA molecular size marker (1.0-kb). Appearance of new bands (a), disappearance of normal bands (b), decrease in band intensities (c) and increase in band intensities (d).

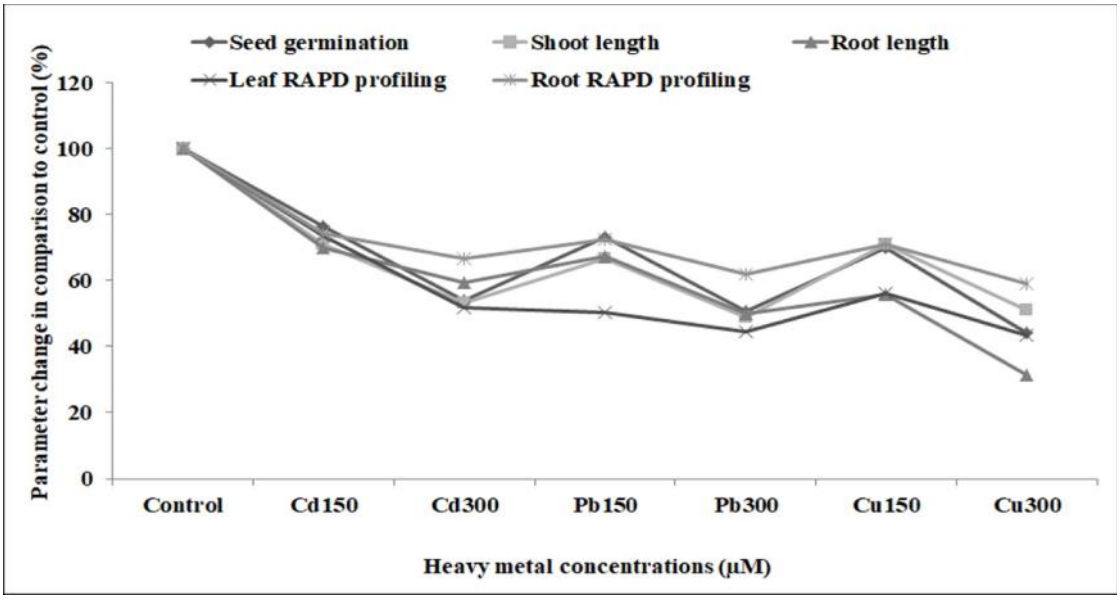

Figure 3. Comparison of root and leaf RAPD profiling (GTS), seed germination, shoot length and primary root length in pea seedlings exposed to heavy metal treatments at different concentrations after 12-day exposure

DISCUSSION

In the present study, the genotoxic effects of Cd, Cu and Pb heavy metals on the germination of the pea seeds were determined as well as shoot and primary root length; and the RAPD assay was performed in the roots and leaves as a molecular parameter. The heavy metal treatments caused significant inhibitions in the germination of the seeds, and in the shoot and primary root length when compared with their respective control. These results are in agreement with the literature about pea (Fusconi *et al.,* 2006, 2007; Hattab *et al.,* 2009; Malecka *et al.,* 2012) as well as with the data reported on other plant species which subjected to heavy metals (Al-Qurainy *et al.,* 2009; Körpe and Aras, 2011; Ahmad *et al.,* 2012).The inhibition in growth is the main response to heavy metal toxicity by higher plants (Malecka *et al.,* 2012). Cd shows high affinity to the -SH groups, zinc and other metal ions are detached from their connection sites. Therefore, it may cause the change and inhibition of enzymes that play roles in various metabolic processes (Clemens, 2006). As a result of these metabolic disorders, it was reported that Cd decreased growth in the root and shoot in plants (Sanita di Toppi and Gabbrielli, 1999). Körpe and Aras (2011) investigated the effects of Cu treatment at various concentration on *Solanum melongena*, and reported that heavy metal treatment caused serious DNA damage in the majority of the cells, and might inhibit cell elongation and cell division in the roots of seedlings. In another study, it was suggested that one of the reasons for the inhibition in the root growth of pea that were exposed to Pb and Cd treatments might be disturbed water and nutrient balance (Piechalak *et al.,* 2010).

In higher plants, heavy metals affect the physical and chemical processes and encourage the formation of secondary metabolites which are part of plant defense system (Mithöfer *et al.,* 2004). The reactive oxygen species (ROS) such as $^{\cdot}O_2$, H_2O_2 and $^{\cdot}OH$ are generally produced under biotic and abiotic stress, and have a strong oxidizing activity that may attack any type of biomolecules (Wojtaszek, 1997). When plants are exposed to the ions of certain heavy metals, free radicals diverge the balance of the metabolism to accumulate H_2O_2 (Mithöfer *et al.,* 2004). Reactive oxygen species induces DNA damages like single strand breakage, double strand breakage, base degradation and cross linkage proteins (Imlay and Linn, 1986).

Many researches were conducted in the past to analyze the toxic effects of heavy metals on *Pisum sativum.* In some of these studies, the effect of Cd on mitotic activity in the apical meristem, mitotic aberrations and microtubule pattern in the roots of pea were investigated (Fusconi *et al.,* 2006, 2007). In another study, Cd and Cu-induced DNA damage in the leaves and roots of pea was determinedby comet-assay (Hattab *et al.,* 2009). To the best of our knowledge, the genotoxic effects of heavy metals in pea were examined with the RAPD analysis for the first time in the current study.

In this study, when compared with the control, variations in appearance of new bands, disappearance of normal bands and band intensity in RAPD patterns were determined in the leaves and roots of the seedlings which exposed to heavy metal. DNA damage (modified or oxidized bases, bulky adducts, DNA strand breaks etc.), nucleotide base changes and/or chromosomal alterations which induced by genotoxic agents could lead to band loss (Wolf *et al.,* 2004; Zhang *et al.,* 2016). Additionally, when DNA polymerase meets a DNA adduct, result of such as possibilities the bypass and blockage which will cause the disappearance of bands (Atienzar and Jha, 2006). The appearance of new RAPD bands may be related to alteration in some oligonucleotide priming sites by reason of large deletions (changing annealing sites), and/or mutations (new annealing sites) (Atienzar *et al.,* 1999).The number of the total appearance of new bands and disappearance of normal bands in the leaves and roots at high concentration was found to be more than in low concentration heavy metal treatments in this study. In addition, in all heavy metal treatments, it was also determined that the number of the disappearance of normal bands was more than the number of the appearance of new bands. Similar evidences reported by Enan (2006) and Cenkci *et al.* (2009).

High concentration of heavy metal treatments resulted more changes in total band intensity (increase and decrease in band intensity) in the leaves and roots of pea seedlings. Heavy metal-induced DNA damage might inhibit polymerization of DNA and reduce the intensity of some RAPD amplicons, whereas DNA modifications increasing the pairing activity of the primer with the DNA may be a probable reason for the increment in intensity of some RAPD amplicons (Lee *et al.,* 2007).

The decrease detected in the GTS values depending on the concentration was observed in the leaves and roots in all the heavy metals used in the study. Similar dose-dependent results were reported in studies in which the effects of different heavy metals on different plant species were investigated with RAPD assay (Liu *et al.,* 2007; Gjorgieva *et al.,* 2012). However, the increase in the polymorphism rates and the decrease in the GTS values were found be more in the roots as compared with leaves as a result of heavy metal treatments. Hattab *et al.* (2009) showed that Cd accumulated in the roots at a rate of 45-fold more and Cu accumulated in the roots at a rate of 15-fold more when compared with the leaves in *Pisum sativum* plants. Malecka *et al.* (2008) examined the Pb accumulation level in *Pisum sativum*, and determined that the most part of the Pb was accumulated in the roots, and only a minor part of the metal (5-7%) was transported to above ground parts of the plant. The correlation between the heavy metal content in tissues and heavy metal-

induced DNA damage was shown with the RAPD assay in bean (Cenkci *et al.,* 2009). In addition, Gichner (2003) determined that the catalase activity, the principal H_2O_2-scavenging enzyme, was more in the leaves of tobacco plant when compared with the roots. The high amounts of catalase and other enzyme contents that inactivate ROS in the leaves may prevent the reaction of the heavy metal-induced ROS with DNA (Hattab *et al.,* 2009). The agreement between the GTS values and the other parameters (seed germination, shoot length and primary root length) that were investigated shows the efficiency of RAPD assay in detecting the DNA damage resulted from toxic pollution.

Conclusion: Recently, in monitoring the environmental effects of pollutants, DNA-based techniques are commonly used in genotoxicity studies. In the present study, it was determined that Cd, Pb and Cu heavy metals showed concentration and tissue-dependent genotoxic effects in the leaves and roots of the seedlings of *Pisum sativum* by using RAPD technique. RAPD assay may detect the temporary DNA changes even at low concentrations of pollutants; and for this reason, is known to be more sensitive than the classical tests.

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