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GENOTOXICITY ASSESSMENT OF HEAVY METALS (Zn, Cr, Pb) ON STRAWBERRY PLANTS USING RAPD ASSAY

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ABSTRACT

The aim of the present study is to assess DNA damage in leaves of strawberry (*Fragaria* × *ananassa* Duch.) seedlings treated with different heavy metals (zinc, chromium, lead) using random amplified polymorphic DNA (RAPD) assay and analysis of total soluble protein content. For this purpose, strawberry seedlings were treated with 400 and 800 µM Zn, Pb and Cr for 7 days. Thirty four RAPD primers produced 218 bands at molecular weight ranging from 183 bp to 5180 bp. Compared with control, RAPD patterns of heavy metal exposed groups showed differences in band loss, gain of new bands and increase and decrease of band intensity. The highest polymorphism rate (32.11%) was observed in 800 µM Pb applied strawberry seedlings. To evaluate the alterations in RAPD profiles qualitatively, genomic template stability (GTS) was performed and the values were 75.08% and 69.59% for 400 and 800 µM Zn treatments, 74.52% and 68.60% for 400 and 800 µM Cr treatments, 70.11% and 63.78% for 400 and 800 µM Pb treatments, respectively. Total soluble protein content in heavy metal-treated groups also showed a similar correlation to GTS values. RAPD analyses are useful biomarker assays to determine the genotoxicity induced by environmental pollutants such as heavy metals in plant model systems.

KEYWORDS:
Biomarker, DNA damage, genomic template stability, total soluble protein

INTRODUCTION

Heavy metal toxicity is a primary threat for environment and human health through bioaccumulation in plant products and the food chain [1]. In recent years, studies have focused on measuring levels of contaminants in tissues and environmental samples, as well as on understanding the mechanism of common contaminant toxicity [2, 3, 4]. Impact assessment of pollutants in eco-genotoxicology is critical important issue [5] and plants, as bioindicators, offer a unique model for monitoring DNA damage and genotoxic stress caused by environmental genotoxins [6]. Heavy metals induce a certain number of cellular stress responses and leading excess production of reactive oxygen species (ROS), which promotes genotoxicity by damaging cellular components such as DNA, proteins and membranes [7]. Oxidative stress is capable of producing many modifications in DNA such as base and sugar lesions, chain breaks, base-free sites and DNA-protein cross-links [8]. With the use of DNA-based techniques developed in recent years, the effects of genotoxic chemicals on DNA can be directly measured precisely in a short time [9]. One of such techniques is the random amplified polymorphic DNA (RAPD) it is possible to detect the nucleotide sequence polymorphisms that are randomly distributed to entire genome, in coding and non-coding regions, as well as in single copy or repetitive sequences [6, 10, 11].

Strawberry (*Fragaria* × *ananassa*) is the most commonly consumed berry crop in the world. However, biomarkers are needed to assess the effects of heavy metals on strawberry, cultivation area of which is increasing all around the world [12]. To our knowledge, this is the first study that investigates the genotoxic effect of heavy metals in strawberry plants. The aim of the present study is to screen genome-wide DNA alterations induced by different heavy metals (zinc, chromium, and lead) in the leaves of strawberry seedlings by using RAPD assay and to analyze the correlation between changes in RAPD profiles and total soluble protein content.

MATERIALS AND METHODS

In the present study, strawberry (*Fragaria* × *ananassa* Duch.) cultivar "San Andreas" seedlings were used as plant material. Strawberry seedlings obtained in 3-leaf stage were grown in plastic pots containing peat and perlite (ratios 1:1). Strawberry seedlings that reached up to 5-leaf stage at the end of 2 weeks were watered with half-strength Hoagland solutions containing zinc sulfate [$ZnSO_4.7H_2O$], potassium dichromate ($K_2Cr_2O_7$) and lead (II) nitrate [$Pb(NO_3)_2$] at concentrations of 400 and 800 µM for 7 days. Concentrations were selected as similar to the concentrations used in previous study [2]. The seedlings were treated with heavy metal containing

½ Hoagland solutions for a total of three times as 20 ml at one-day intervals and samples were collected at 48th h after the last treatment. Non-exposed seedlings (watered with metal-free half-strength Hoagland solution) were used as controls. The plastic pots were incubated in a growth chamber for 16 h-light (with light intensity approx. 170 µM m^{-2} s^{-1}) and 8 h-dark photoperiod. During the growth period, the temperature was 20/17°C day / night; humidity was 70–75%. At the end of the 7th day, the leaves of harvested seedlings were separated and stored at −80°C until the total soluble protein and RAPD assays were carried out. Each experiment was performed in triplicates and each replicate contained seedlings with equal size and numbers (20 plants per replicate).

The total soluble proteins were extracted from 500 mg of frozen leaf samples and determined as described previously [13]. Experimental results from triplicates were analyzed and expressed as the mean ± standard error (SE). Analysis of variance (ANOVA) was performed, and Duncan's multiple range tests at 0.01 confidence level were applied to compare significant differences between the control and each treated group.

Genomic DNA was extracted from frozen leaf tissues using cetyltrimethylammonium bromide (CTAB) protocol [14]. DNA concentration and purity (OD260 / OD280) were measured with NanoDrop (MaestroGen, USA). Following the measurement, all DNA samples were diluted with distilled H_2O to 5 ng μL^{-1}. An initial screening of 50 10-mer random primers (Operon Technologies, USA) was performed and among them, 38 primers amplified clean and repeatable bands for RAPD assay. Sequences of 38 primers used in the study are given in Table 1. RAPD-PCR was performed in 15 µL reaction mixture containing 1.5 µL 10X Taq buffer (100 mM Tris-HCl pH 8.8, 500 mM KCl, 0.8% Nonidet 40), 1 µL primer (10 µM), 2.4 µL $MgCI_2$ (25 mM), 1 µL dNTP (10 mM), 0.25 µL Taq DNA polymerase, and 25 ng of genomic DNA as template. Amplifications were carried out in a thermocycler (BioRad T100, USA) programmed for initial denaturation step (1 min at 94°C); 45 three-step cycles of denaturation (1 min at 94°C), annealing (1 min at 36°C), and extension (2 min at 72°C); followed by final extension step 10 min at 72°C. A negative control was run with each sample set. The amplified products were loaded in 2% (m/v) agarose gel containing 1% (v/v) safe DNA gel stain (Invitrogen, USA) and run at 80 V for 1.5 h. Gel images were captured with imaging system Fusion FX7 (Vilber Lourmat, Germany) under UV light and molecular sizes of the amplicons were determined using Fusion-CAPT-Software 16.07. A 1.0-kilobase (kb) DNA Ladder (Thermo Scientific, Germany) was loaded as marker in each gel. Marker bands on all gels were visualized from top to down as 10000, 8000, 6000, 5000, 4000, 3500, 3000, 2500, 2000, 1500, 1000, 750, 500, and 250 base-pair (bp). Reproducibility of RAPD patterns were confirmed by repeating all amplifications at least 3 times.

Changes in the RAPD profile were expressed as Genomic Template Stability (GTS), which is a qualitative measurement showing the obvious changes in the number of RAPD profiles. GTS (%) was calculated for each primer using the formula: $GTS = (1 - a / n) \times 100$, where "a" is the number of polymorphic bands detected in each treated sample and "n" is the total number of control bands. Changes in these values were calculated as a percentage of their control (set to 100%) to compare the sensitivity of parameters (GTS, total soluble protein).

TABLE 1
Sequences of 38 primers used in this study

Primer no	Primer name	Primer sequence (5'→3')	Primer no	Primer Name	Primer sequence (5'→3')	Primer no	Primer Name	Primer sequence (5'→3')
1	OPA-01	CAGGCCCTTC	14	OPB-18	CCACAGCAGT	15	OPM-09	GTCTTGCGGA
2	OPA-02	TGCCGAGCTG	15	OPB-20	GGACCCTTAC	16	OPM-10	TCTGGCGCAC
3	OPA-11	CAATCGCCGT	16	OPC-04	CCGCATCTAC	17	OPM-11	GTCCACTGTG
4	OPB-01	GTTTCGCTCC	17	OPC-05	GATGACCGCC	18	OPM-12	GGGACGTTGG
5	OPB-04	GGACTGGAGT	18	OPD-08	GTGTGCCCCA	19	OPM-13	GGTGGTCAAG
6	OPB-05	TGCGCCCTTC	19	OPH-18	GAATCGGCCA	14	OPM-15	GACCTACCAC
7	OPB-06	TGCTCTGCCC	20	OPM-01	GTTGGTGGCT	33	OPM-16	GTAACCAGCC
8	OPB-07	GGTGACGCAG	21	OPM-02	ACAACGCCTC	34	OPM-17	TCAGTCCGGG
9	OPB-08	GTCCACACGG	22	OPM-03	GGGGGATGAG	35	OPM-18	CACCATCCGT
10	OPB-10	CTGCTGGGAC	23	OPM-04	GGCGGTTGTC	36	OPM-19	CCTTCAGGCA
11	OPB-11	GTAGACCCGT	24	OPM-05	GGGAACGTGT	37	OPW-01	CTCAGTGTCC
12	OPB-15	GGAGGGTGTT	25	OPM-06	CTG GGCAACT	38	OPW-05	GGCGGATAAG
13	OPB-17	AGGGAACGAG	26	OPM-07	CCGTGACTCA			

TABLE 2
Effects of heavy metals on total soluble protein content in strawberry leaves after 7 days of treatment

Heavy metal concentrations (µM)	Total soluble protein content (mg g^{-1} fresh weight)*
Control	28.17 ± 0.15 [a]
Zn 400	26.09 ± 1.17 [b]
Zn 800	24.88 ± 0.07 [c]
Cr 400	25.78 ± 0.08 [b]
Cr 800	24.39 ± 0.07 [c]
Pb 400	24.60 ± 0.11 [c]
Pb 800	20.91 ± 0.42 [d]

*Different letters present significant differences at $P < 0.01$ according to Duncan's multiple range tests. Values are given as mean ± SE (n = 3).

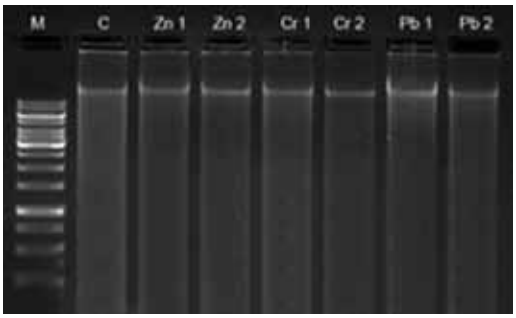

FIGURE 1
Genomic DNA samples extracted from the leaves of heavy metal treated (Zn 1 : 400 µM Zn, Zn 2 : 800 µM Zn, Cr 1: 400 µM Cr, Cr 2 : 800 µM Cr, Pb 1 : 400 µM Pb, Pb 2 : 800 µM Pb) and untreated (C: control) seedlings using modified CTAB DNA extraction protocol. M : DNA molecular size marker (1.0-kb)

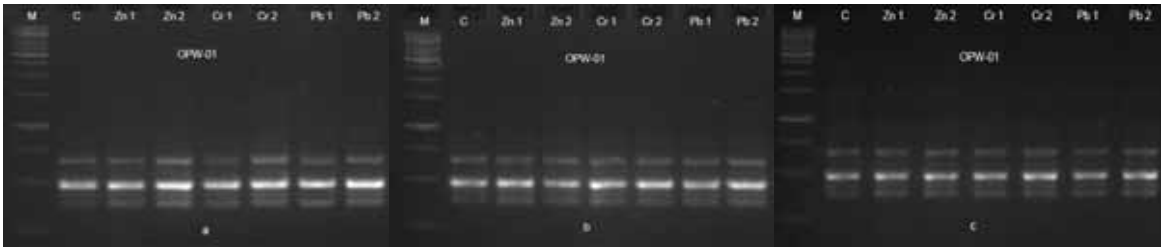

FIGURE 2
Reproducibility of RAPD profiles in DNA samples extracted from strawberry leaves. OPW-01 primer generated the same band pattern in triplicates (a, b, c) of control and heavy metal treated samples: C : control, Zn 1 : 400 µM Zn, Zn 2 : 800 µM Zn, Cr 1 : 400 µM Cr, Cr 2 : 800 µM Cr, Pb 1 : 400 µM Pb, Pb 2 : 800 µM Pb. M : DNA molecular size marker (1.0-kb).

RESULTS

Total soluble protein content of strawberry seedlings treated with Zn, Cr and Pb at different concentrations and control are given in Table 2. Total soluble protein content of heavy metal-exposed group, statistically decreased compared to control samples ($P < 0.01$). 800 µM Pb treatment affected the total soluble protein content of the strawberry leaves more than the other treatments (Table 2).

Suitability of modified CTAB method for genomic DNA extraction from strawberry leaves was assessed based on the integrity and purity of genomic DNA samples. The purity indexes of the extracted DNA samples were between 1.6–1.8 and DNA concentrations ranged from 220 to 280 μg^{-1} fresh weight, approximately. The quality of the DNA was checked by gel electrophoresis and single band was observed in all extracted DNA samples (Figure 1). In the present study, extracted genomic DNA always gave same banding pattern with same primer, thus results supported the consistency of the RAPD assay (Figure 2).

Fifty 10-mer random primers were tested for screening changes in the strawberry genome and among them, 38 primers (76%) generated clear and

reproducible results. Thirty four primers (89.47%) out of mentioned 38 primers showed different RAPD profiles in control and heavy metal exposed groups while there were no differerences in the rest 4 primers (10.53%): OPM-02, OPM-16, OPW-01 and OPW-05.

Thirty eight primers amplified a total of 218 DNA fragments between 183 bp (OPM-07) and 5180 bp (OPB-01). According to the results, RAPD patterns of the control and heavy metal-exposed samples showed differences in the size, number (loss of normal bands and / or appearance of new bands), and intensities of amplified DNA fragments. RAPD profiles of four selected primers (OPB-15, OPM-06, OPM-07 and OPM-12) are given in Figure 3. Tables 3 and 4 summarize the changes detected in RAPD profiles of the leaves of strawberry seedlings exposed to heavy metals. Each primer produced 2-12 bands and the average number of bands per primer was 6.65. Amplified band sizes in control samples ranged from 183 bp (OPM-07) to 5180 bp (OPB-01). Among the primers used in the present study, OPM-17 was the primer giving the highest number of polymorphic bands (9 polymorphic bands) ranging from 620 bp to 2500 bp, while OPB-06, OPB-18, OPC-04, OPM-03 and OPM-10 primers gave only 1 polymorphic band ranging from 457 bp to 2380 bp.

Total band changes in terms of band gain and loss for 400 µM Zn, 800 µM Zn, 400 µM Cr, 800 µM Cr, 400 µM Pb and 800 µM Pb treatments are 51, 55, 55, 64, 57 and 70, respectively. Comparing all the heavy metal-exposed groups with the control group, the maximum band loss (35 bands) was observed in 400 µM Cr treatment.

On the other hand, the highest number of extra bands (42 bands) was observed in 800 µM Zn and Pb treatments. Maximum number of new RAPD bands was detected in OPA-11 primer (4 bands, 300–903 bp) for 400 and 800 µM Zn treatments; and OPM-17 primer (4 bands, 720–2200 bp) for 400 and 800 µM Zn, Cr and Pb treatments (Table 3). In the present study, some polymorphic bands were observed only at low concentrations (400 µM) or only at high concentrations (800 µM), while some polymorphic bands were specific to heavy metal (Table 3).

In the RAPD profiles of seedlings, exposed to 400 and 800 µM Zn, Cr and Pb treatments, decreases and increases in band intensities were observed in 38 primers (Table 4). Total band intensity changes (increased and decreased band intensity) of 400 µM Zn, 800 µM Zn, 400 µM Cr, 800 µM Cr, 400 µM Pb, and 800 µM Pb treatments were recorded as 30, 44, 31, 38, 29, and 35, respectively (Table 4). Figure 3 shows the band intensity changes observed in the RAPD profiles of OPM-06, OPM-07 and OPM-12 primers of heavy metal-treated seedlings.

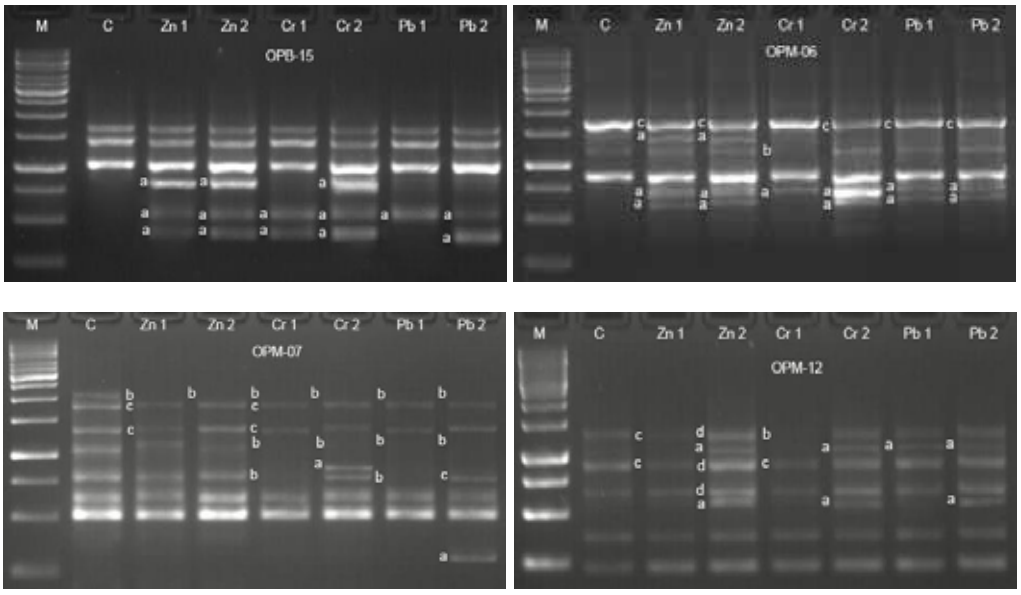

FIGURE 3
RAPD profiles generated by OPB15, OPM-06, OPM-07 and OPM-12 primers with DNA samples extracted from control and heavy metal treated leaves of strawberry seedlings. C: control, Zn 1 : 400 µM Zn, Zn 2 : 800 µM Zn, Cr 1 : 400 µM Cr, Cr 2 : 800 µM Cr, Pb 1 : 400 µM Pb, Pb 2 : 800 µM Pb; appearance of new bands (a), disappearance of normal bands (b), decrease in band intensities (c) and increase in band intensities (d). M : DNA molecular size marker (1.0-kb)

TABLE 3
Changes in the RAPD profiles (molecular sizes – bp) related to the selected concentrations of Zn, Cr and Pb compared to control for all used primers in the leaves of stawberry seedlings

Primers		Zn (μM)		Cr (μM)		Pb (μM)	
		400	800	400	800	400	800
OPA-01	+	0	0	797	0	1075	1075
	−	265	265	265	265	265	265
OPA-02	+	0	1200	0	0	0	1822
	−	0	0	0	0	0	0
OPA-11	+	903; 550; 410; 300	903;550; 410; 300	903;550; 300	903; 550; 300	903; 550; 300	903; 550; 300
	−	0	0	0	0	0	0
OPB-01	+	4250	4250	4250	4250	4250	4250
	−	848	848	848	848	848	848
OPB-04	+	0	0	0	0	1968; 1035	1968; 1035
	−	0	0	0	0	370	0
OPB-05	+	1250; 950	1250; 1000;950	950	1250; 950	1250	0
	−	0	0	0	0	0	0
OPB-06	+	0	0	0	1973	0	1973
	−	0	0	1245	0	0	0
OPB-07	+	2037	0	0	0	0	0
	−	0	0	0	0	0	720; 620; 440
OPB-08	+	0	950	0	950	950	950
	−	450	0	450	450	450	450
OPB-10	+	0	0	0	569; 510	0	569; 510
	−	0	0	0	3315	673	673
OPB-11	+	0	500	0	500	2190; 500	2190; 500
	−	0	0	0	0	0	0
OPB-15	+	650;390; 275	650; 390; 275	390; 275	650; 390; 275	390	390; 275
	−	0	0	0	0	0	0
OPB-17	+	1045	0	0	0	0	0
	−	0	0	0	0	0	2846
OPB-18	+	474	474	474	474	474	474
	−	0	0	0	0	0	0
OPB-20	+	0	0	0	0	1530; 1330; 835	1530; 1330; 835
	−	0	0	0	0	0	0
OPC-04	+	500	0	0	0	0	0
	−	0	0	0	0	0	0
OPC-05	+	0	1055	0	0	0	1055
	−	1159; 945	0	1159	1159	0	0
OPD-08	+	0	0	0	0	0	0
	−	1902; 1443; 1046; 405	1902; 1443; 1046	1902;1443; 1046; 405	1902; 1443; 1046; 405	1902; 1443; 1046	1902; 1443; 1046
OPH-18	+	0	604	0	0	0	0
	−	505	0	505	0	505	0
OPM-01	+	600	1460; 600; 550	1460; 600; 550	1460; 600; 550	1460; 600; 550	1460; 600
	−	0	0	0	2074	0	0
OPM-03	+	0	0	0	0	2380	2380
	−	0	0	0	0	0	0
OPM-04	+	870	870	870	870	0	0
	−	2020; 1900; 550	0	2020; 1900; 1700; 550; 430	1900; 1700; 550	1900; 550	1900; 550
OPM-05	+	0	0	568	568	1400	1400
	−	759	2443; 759	759	2443; 2163; 759	0	2443
OPM-06	+	1422,735; 643	1422, 735; 643	735	735, 643	735, 643	735, 643
	−	0	0	1245	0	0	0
OPM-07	+	0	0	0	885	0	339
	−	2315	2315	2315; 1172; 760	2315; 1172	2315; 1172; 760	2315; 1172
OPM-09	+	1065; 980	3350; 980; 750	980	980;750	980	3350; 980
	−	2730; 1562	2730; 1562	2730; 680; 590	2730; 1562	2730; 1562	2730; 590
OPM-10	+	0	820	0	820	0	0
	−	0	0	0	0	0	0
OPM-11	+	0	1245; 1000	0	832; 446	0	0
	−	3800;1440	0	1440; 550	0	1640;1440; 550	1640; 1440; 550
OPM-12	+	0	1150;570	0	1150; 570	570	1150; 570
	−	0	0	1380	0	0	0
OPM-13	+	525	0	525	525	2750; 525	2750; 525
	−	1860; 420	0	2350; 1860; 1025; 605; 420	2350; 1860; 1025; 605; 420	1860; 420	1860; 420
OPM-15	+	0	732; 375	0	375	375	1471; 375
	−	0	0	0	0	0	0
OPM-17	+	2200; 870; 720; 620	2200; 1230; 870; 720	2200; 1230; 870	2200; 1230; 870; 720	2200; 1230; 870	2200; 1230; 870; 620
	−	2500; 1900	2500; 1900	1900; 1030	1030	1900; 1030	1900; 1030
OPM-18	+	0	3000; 803; 385	0	0	3000	3000
	−	0	0	1420	0	0	1420
OPM-19	+	0	508	0	0	0	2160
	−	1060; 750	750	1060; 750	1060; 750	1060; 750	1060; 750

"+" appearance of new DNA bands and/or "–" disappearance of DNA bands

TABLE 4
RAPD profile changes, average polymorphism (%) rates and genomic template stability (%) values as detected with all used primers in the leaves of seedlings exposed to different heavy metals for 7 days.

Heavy metal treatments (µM)	RAPD profile changes*				Polymorphism (%)	GTS (%)
	a	*b*	*c*	*d*		
Control	-	-	-	-	0	100
Zn400	26	25	19	11	23.39	75.08
Zn800	42	13	5	39	25.22	69.59
Cr400	20	35	16	15	25.22	74.52
Cr800	36	28	7	31	29.35	68.60
Pb400	32	25	15	14	26.14	70.11
Pb800	42	28	18	17	32.11	63.78

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

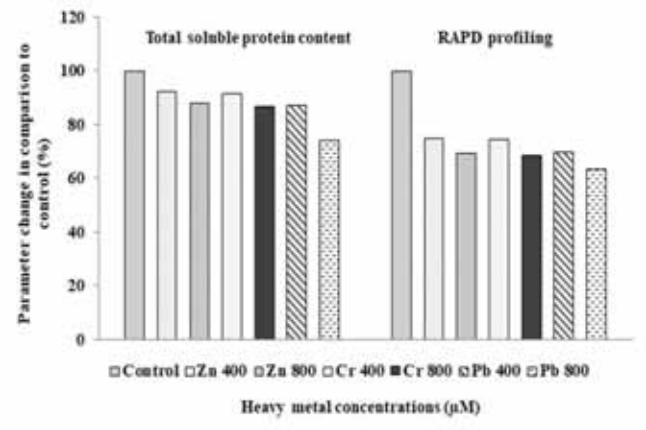

FIGURE 4
Comparison of RAPD profiling (GTS) and the total soluble protein content in leaves of strawberry seedlings exposed to heavy metal treatments at different concentrations for 7 days

Higher concentrations of metal treatments (800 µM Zn, Cr, and Pb) showed greater number of changes in RAPD band intensities compared to lower concentrations (400 µM Zn, Cr, and Pb). Different polymorphic bands in different primers were detected at certain concentrations of heavy metal treatments. Polymorphism values for 400 µM Zn, 800 µM Zn, 400 µM Cr, 800 µM Cr, 400 µM Pb, and 800 µM Pb treatments were 23.39%, 25.22%, 25.22%, 29.35%, 26.14%, and 32.11%, respectively (Table 4).

GTS values of treated samples compared to control samples. The calculated GTS values are given in Table 4. Comparing to the control samples after 7 days of treatment, the mean GTS values were 75.08%, 69.59% for 400, and 800 µM Zn treatments; 74.52%, 68.60% for 400 and 800 µM Cr treatments; 70.11%, 63.78% for 400, and 800 µM Pb treatments, respectively. While similar mean GTS values (75.08%, 74.52% and 70.11%, respectively) were obtained for 400 µM Zn, Cr and Pb treatments; the lowest value (63.78%) resulted in 800 µM mg L^{-1} Pb treatment (Table 4).

Comparison of GTS values and total soluble protein contents are given in Figure 4. GTS values and the total soluble protein contents in the leaves of the strawberry seedlings exposed to heavy metals were correlated.

DISCUSSION

Heavy metals can directly or indirectly affect human health together with aquatic flora and fauna; and cause harmful effects due to certain properties such as high solubility, long half-life period, nonbiodegradable nature, and tendency of bioaccumulation and bio-magnification [15]. Structural similarity of Cr to sulphates and phosphate ions allows the molecule to easily enter the cell by mimicking these ions [16]. It causes structural genetic lesions, breaks in the DNA strand, unusual DNA-protein cross-links and oxidation of the bases by reducing within the cell [17]. Zou et al. [18], evaluated the effects of Cr (VI) on root cell growth and cell division in *Amaranthus viridis* root tips. Increased Cr (VI) concentration was shown to reduce the mitotic index, as well as to increase the C-mitotic frequency, causing chromosomal morphology changes such as chromosomal bridges, anaphase bridges and chromosomal adhesions. Zinc is an essential nutrient and is needed in very small quantities for both plants and animals; on the other hand, accumulation of zinc in the soil is toxic to plants and microorganisms [19]. Truta et al. [20] investigated the effects of Zn (II) on root meristems of *Hordeum vulgare* and found that zinc treatments led to formation high level chromosome aber-

rations. It is also thought that zinc inhibits DNA repair processes in mammals by inhibiting O6-alkylguanine-DNA-alkyl transferase and DNA ligase I activities and indirectly enhances the genotoxic effects of heavy metals [21]. Lead is a common pollutant in the environment due to many industrial activities. Pb accumulation in the atmosphere and in the soil can be dangerous for all organisms, including plants [22]. It is known that nitrate or iodine salts of Pb cause C-mitosis, inhibit root development, and decrease mitotic activity. Studies in plants belonging to the genus *Allium* have indicated that lead has various genotoxic and clastogenic effects such as formation of anaphase bridges and diplochromosomes, as well as dissociation and fragmentation of chromosomes [23].

In the present study, the genotoxic effect of Zn, Pb, and Cr treatments on strawberry seedlings at 400 and 800 µM concentrations were evaluated by changes in RAPD band profile: band loss, new band formation and increase and decrease of band intensity in comparison with the control group. RAPD assay has been used successfully to assess DNA damage induced by heavy metals [24]. The major advantages of the RAPD assay are its rapidity, lack of radioactivity, lack of enzymatic degradation of PCR products, application to any organism, and potential detection of wide range of DNA damage [25].

DNA damage in the plant genome exposed to the stress factor is reflected as differences in band profiles [6]. In the present study, Cr, Zn and Pb heavy metal treatments in the leaves of strawberry seedlings introduced a total of 120 new bands at high concentration (800 µM) and 78 new bands at low concentration (400 µM). New RAPD amplicons could originate from new annealing events caused by mutations such as large deletions and/or homologous recombination [26]. In a study by Gjorgieva et al. [5], RAPD assay was used to investigate the genotoxic effects of different heavy metals in *Phaseolus vulgaris*. As a result of the study, they reported that the total number of new bands was higher in plants exposed to high concentration of heavy metals compared to the low concentration. Similar results have been reported in another study [25] and these results have supported findings of the present study.

In the present study, high concentration (800 µM) of heavy metal treatment resulted in a total of 69 bands and a total of 85 bands loss at the low concentration (400 µM) treatment in the strawberry seedlings. DNA damage such as modified or oxidized bases, single-strand breaks, double-strand breaks, bulky adduct, point mutations and/or complex chromosomal rearrangements induced by genotoxic chemicals could lead to disappearance of normal bands [5]. In contrast to our work, previous studies in which the genotoxic effects of different heavy metals on different plant systems have shown that treatments at higher concentrations were associated with greater loss of bands compared to lower concentrations [27]. However, in the present study, high-concentration of Pb resulted in more band loss (28 bands) than low-concentration of Pb treatment (25 bands).

After 7-day treatment, heavy metals (Zn, Cr, Pb) at different concentrations showed similar polymorphism rates in the leaves of strawberry seedlings. However, dose-dependent effect of Pb treatment on DNA was more prominent than that of Cr and Zn. Cenkci et al. [25] determined polymorphism rate in the leaves and root tissues of *Phaseolus vulgaris* exposed to Cr and Zn as 30% and 25.3%, respectively.

The number of RAPD primers used for studies of genotoxicity of heavy metals on different plant systems using the RAPD assay generally ranged from 1 to 20. For example, in order to investigate the genotoxic effects of aluminum (Al) and nickel (Ni) heavy metals in *Phaseolus vulgaris* plant, 10 primers were used [28]. In another study, 20 primers were used to determine cadmium (Cd)-induced DNA damage in *Cuminum cyminum* [29]. One primer was used in the study in which genotoxic effects of Boron (B) on *Triticum aestivum* were investigated [30]. In the current study, more primers (50 primers tested and 38 primers gave reproducible results) than the other studies were tested. In this way, the aim was to evaluate heavy metal-induced genotoxicity in more different locations of the genome comprehensively.

In the present study, higher concentrations of heavy metal treatments (117 bands) caused more changes in total band intensity (increase and decrease in band intensity) more than the treatments at lower concentration (90 bands). Variations such as mutations resulting from heavy metal exposure, genomic rearrangements and structural modifications affect the polymerization of DNA in the PCR reaction. As a result, increase and decrease of RAPD band intensity were observed [26, 31].

Changes observed in RAPD profile are reflected as modifications in genomic template stability (GTS) and GTS values can be directly compared with changes in biochemical parameters (such as total soluble protein content). In the present study, similar GTS values (75.08%, 74.52% and 70.11%) were observed for 400 µM Zn, Cr and Pb treatments while the lowest GTS value (63.78%) was at 800 µM Pb treatment. Results showed that strawberry seedlings are more susceptible to genotoxic effects of Pb at high concentrations, while Zn and Cr metals have less effect on DNA integrity in terms of concentrations used in the study. Erturk et al. [32] investigated the genotoxic effect of 5, 10, 20, 40 mM Zn treatments on *Zea mays* for 7 days using RAPD assay and detected average GTS values as 67.5%, 58.8%, 56.8% and 52%, respectively. Pb treatment at a concentration of 800 µM reduced the total amount of soluble protein in the strawberry leaves more than other treatments (74.22%). Liu et al. [33] studied

cadmium (Cd)-induced DNA changes in *Hordeum vulgare* by determining protein content and using RAPD assay. The result of the study was similar to the results of present study, and the application of Cd resulted in significant decrease in protein levels of barley plants.

CONCLUSIONS

Strawberry is an important part of our diet and is a significant source of micronutrients such as antioxidant phenolics. Probable genotoxicity due to metal contamination should not be neglected, especially for plants that are used as food and have medicinal properties. Random amplified polymorphic DNA (RAPD) assay is a fast and inexpensive method that allows for the first screening to assess toxicity when conventional toxicology data is limited or insufficient.

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