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Cadmium and lead- induced genotoxicity in date palm (*Phoenix dactylifera* L.) cv. Barhee

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Abstract

Genotoxicity of Cadmium (Cd) and Lead (Pb) heavy metals were investigated in date palm (*Phoenix dactylifera* L.) whole plant in laboratory under controlled conditions. The genotoxic effects were evaluated using protein profile and ISSR molecular markers. Two concentrations of cadmium and lead were selected as 3 and 9 mg/kg for Cd; 100 and 276 mg/ kg for Pb. The results clearly showed that the Cd at two examined concentrations and Pb at low concentration (100 mg/kg) did not induce any changes in genetic materials in terms of protein patterns and ISSR markers when compared with untreated date palm plants (control plants). The high concentration of Pb (276 mg/kg) led to appearance of a new protein fragment (18 KDa), as well as, a disappearance of the fragment of 33 KDa which observed in all treatments. ISSR analysis and Dendrogram revealed the separation of Pb at high concentration in one cluster with the highest genetic distance (0.2 according to Nie and Li's index) than control treatment. In addition, results indicated that genomic template stability (GTS) was significantly affected by Pb at high concentration and reduced to 70% which reflects the genotoxicity of Pb at 276 mg/kg. Our results highlight for the first time the genotoxic effects of Pb at high concentration on date palm genome.

Keywords: Genome template stability; heavy metals; ISSR; protein profile; SDS-PAGE.

Abbreviations: **GD**: genetic distance; **gDNA**: genomic DNA; **GTS**: genome template stability**; ISSR**: inter simple sequence repeat; **KDa**: kilo dalton; **PCR**: polymerase chain reaction; **RAPD**: random amplified polymorphism DNA; **RPM**: revolution per minute; ROS: reactive oxygen species; **RT**: room temperature; **SDS-PAGE**: sodium dodecyl sulfate- polyacrylamide gel electrophoresis; **UPGMA**: unweighted pair group mean average.

Introduction

Environmental pollution has become a worldwide problem causing a severe losses in agriculture; as well as, health hazard. Heavy metals are considered as one of the most important pollutants to the environment because of their toxicity, non-biodegradability, mostly long biological half life (10-30 years) and a wide range of distribution (Duffus, 2002, Pinto *et al*., 2003). Heavy metals can be defined as metals having a density higher than 5 g cm^3 (Weast, 1984). The toxicity of HMs is mainly depends on several factors such as the solubility of the metal, absorbability, transport, its chemical reactivity, pH of the medium and presence of other ions (Das *et al*., 1997).

The toxicity of HMs to plants could be attributed to different mechanisms including inhibition of enzymes by binding their sulfhydryl groups (Choudhury and Panda, 2004); decreasing the relative water content and transpiration by affecting the water imbalance (Krantev *et al*., 2006); increasing the permeability of the plasma membrane (Janicka-Russak *et al*., 2008); inhibition of photosynthesis pigments such as chlorophyll a, b and carotenoides, and increasing the activity of Reactive oxygen species (Abass *et al*., 2016); and increasing the level of lipid peroxidation and producing of malondialdehyde (Zouri *et al*., 2016). Additionally, ROS induce DNA damage either by breaks of DNA strands or inhibition of DNA replication by binding protein involved in replication, resulting in alteration of encoded proteins and lead to a malfunction of protein in plant cells (Lin *et al*., 2007; Nanda and Agrawal, 2016). High levels of HMs pollution have been reported in the soils and leaves of date palm at different areas of Basra governorate/ Iraq recently; the level of cadmium and lead have exceeded the European standard acceptable levels (European Standards, 2006) and reached 9 and 276 mg/ kg for Cd and Pb; respectively (Abass *et al*., 2015, Al-Jabary *et al*., 2016). It's noteworthy that Cd and Pb (and many other HMs) have no known biological functions as nutrients, and are very toxic to plants and microorganisms (Nies, 1999).

In recent years, several studies have been focused on the genotoxicity of heavy metals on plants; different selective and sensitive molecular markers have been developed to assess the genetic instability caused by direct exposure to HMS. Random Amplified Polymorphic DNA (RAPD) assay has been successfully applied in detection of genotoxic effects induced by cadmium and lead in *Phaseolus vulgaris* L.; *Brassica rapa* L., *Zea mays* L.; *Sesbania grandiflora* and *Secale cereale* L. (Enan, 2006; Cenkci *et al*., 2010; Ertuk *et al*., 2012; Malar *et al*., 2014; Ozyigit *et al*., 2016). Inter Simple Sequence Repeat (ISSR) marker has been effectively used in measuring genome template stability (GTS) under the effect of heavy metals pollution depending on the analysis of banding patterns of amplified DNA in treated and control plants, such as in *Eruca sativa* L.; *Pistia stratiotes*; *Viola tricolor* L.; *Plantago* spp. and *Solanum nigrum* L. (Al-Qurainy, 2010; Slomka *et al*., 2011; Neeratanaphan *et al*., 2014; Correia *et al*., 2014; Al Khateeb and Al-Qwasemeh, 2014).

Another non-PCR techniques have been used to measure the DNA damage caused by heavy metals pollution including protein profile analysis in treated *Brassica* species and *P. vulgaris* with Cd and Pb (Iqbal *et al*., 2012; Aldoobie and Beltagi, 2013); Comet assay also

used in evaluation the DNA damage in whole root cells of *Vicia faba* treated with different concentrations of Pb (Pourrut *et al*., 2011).

Date palm (*Phoenix dactylifera* L.) is a member of the Arecaceae family, a dioecious, perennial, monocotyledonous tree and cultivated mainly for their nutritive fruits (Abass, 2013); with an importance role in sustainable agriculture in many countries worldwide including Middle East and north Africa for more than five thousand years (Al-Khayri *et al*., 2015).

To the best of our knowledge; this is the first study with an attempt to evaluate the genotoxic affects of Cd and Pb on the genome template stability of date palm whole plant using both protein profile and ISSR marker.

Materials and Methods

Plant Materials and Heavy Metals treatment

Two years old date palm offshoots of Barhee cultivar derived from tissue culture (to avoid any unnecessary genetic variations; their genetic uniformity was ascertained) were selected. For Pb and Cd treatments; two concentrations were selected according to the study of Al-Jabary *et al*. (2016); the minimum and maximum concentrations were 3 and 9 mg/kg for Cd; respectively; 100 and 276 mg/kg; for Pb; respectively.

The offshoots of date palm were planted in pots; each pots was filled with 5 kg soil, the chemical characteristics of soil was describes as follow: pH= 7.62; electrical conductivity = 5.23 dsm/l; cation exchange capacity= 9 cmole and organic matter = 0.81 %. Soil texture was loam (silt = 45.65; sand = 32.44 and clay = 21.91). All offshoots were subjected to laboratory conditions during the experiment (light density= 10,000 Lux; temperature = 27±2 $^{\circ}$C and relative humidity of 60–70%). Heavy metals treatments were as follow:

Control: non-stressed date palm plants.
Cd1: 3 mg/kg as cadmium chloride.
Cd2: 9 mg/kg
Pb1: 100 mg/kg as lead acetate.
Pb2: 276 mg /kg.

Each heavy metals treatments were applied to plants by irrigation with Reversible Osmosis (RO) water; the chemical properties of irrigated water were: pH = 7.6; EC = 1.3 dsm/l; Ca^{+} = 5.9; Mn^{+} = 15.8; Na^{+} = 19; Cl^{-} = 15 and K^{+} = 1.9 ppm.
The trail was conducted with triplicates for each treatment at the Date Palm Research Centre, University of Basra. Plants were irrigated with heavy metals treatments for 180 days.

Date Palm Protein Extraction and SDS-PAGE electrophoresis.

300 mg of HMs treated date palm fresh leaves was ground in liquid nitrogen and homogenized in 3 ml Tris-HCl buffer (0.1 M, pH 7.5) containing 1 mM phenylmethanesulfonylfluoride (PMSF) at 4 º C. Then centrifuged for 30 min at 13 krpm (Bavei *et al*., 2011). The protocol of Bradford (1976) was followed to measure the protein content in each sample, using Bradford reagent (containing: 100 mg Coomassie Brilliant Blue R-250 in 50 ml 95% ethanol, 100 ml 85% (w/v) phosphoric acid), a crystalline bovine albumin (5–100 µg protein) was used to establish a standard curve at 595 nm.

The concentration of protein in all examined samples was diluted to 40 µg using sterilized distilled water, protein samples were subjected to discontinuous polyacrylamide gel electrophoresis (PAGE) under non-denaturing conditions according to Leammli (1970). Electrophoretic separation was performed at 4 º C for 7 h, using 15% polyacrylamide gels. Staining with commassie brilliant blue and destaining were performed as described in Meyer and Lambert (1965).

ISSR Marker

Date Palm genomic DNA Extraction

The genomic DNA (gDNA) of treated date palm leaves was extracted according to Doyle and Doyle (1990) using CTAB (cetyltrimethyl ammonium bromide) method, briefly as follow:

- 250 mg of leaves were frozen and ground in liquid nitrogen into a fine powder.
- Powder was then mixed with 700 µl of CTAB extraction buffer containing: 20 mM EDTA, 0.1 M Tris-HCl pH 8.0, 1.4 M NaCl, 2% CTAB, and 0.4% b-mercapto ethanol added prior to use.
- The solution was then heated up to 65 ºC for 45 min, and mixed gently by inversion every 15 min.
- Subsequently, a 500 µl of chloroform-isoamylalcohol (24:1) was added to the mixture and gently mixed for 1 min.
- Centrifugation for 1 min at 12 krpm was followed, and 600 µl as supernatant was transferred into a fresh Eppendorf tube containing 500 µl chloroform-isoamylalcohol (24:1).
- 500 µl of the supernatant was transferred into a fresh tube containing 700 µl ice-cold isopropanol, mixture was mixed very well.
- Another centrifugation for 10 min at 12 krpm was followed, the supernatant was removed, and the DNA pellets were washed with 70% ethanol (700 µl).
- Samples were dried at RT.
- Ressuspension of the pellets was followed with 100 µl TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0); and incubated for one hour at 37 º C.

Extracted DNAs were quantified in 1.5% agarose gels to check the integrity of gDNA. Electrophoresis was conducted in a 1X TBE buffer [100 ml 10X TBE (0.89 M Tris base, 0.89M Boric acid, 20 mM EDTA pH 8.0) and 900 ml distilled water] at 60 V for 30 min and then at 120 V for 1.5 h. A 0.5 mg/m of ethidium bromide was used to stain the DNA. The concentrations of gDNA were measured by Nano-Drop spectrophotometer (Bio-Rad,

USA) at A260/280, gDNA templates were diluted to 30 ng/ µl with TE buffer (Abass *et al*., 2017).

ISSR Primer Description and PCR Conditions

Five ISSR molecular markers were ordered from Bioneer- Korea; the full description of each primer was mentioned in Table 1. A total volume of 25 µl reaction was used for polymerase chain reaction (PCR) was carried containing 30 ng template DNA, 1.5 mM $MgCl_2$, 0.32 mM dNTPs, 1X *Taq* DNA polymerase buffer, 10 pmol ISSR molecular marker and 2 units of *Taq* DNA polymerase (iNtRon, Biotechnology Inc., Korea).

PCR reaction was performed in a thermal cycler using the following conditions: denaturation at 95 º C for 3 min; 35 cycles of 1 min denaturation at 95 C, 1 min annealing at 36 º C and 2 min extension at 72 ºC; and a final extension at 72 º C for 7 min.

Data Scoring and Analysis

The precise protein and DNA bands were detected according to the protein molecular weight marker of Promega (10–225 KDa, nine fragments) and DNA marker of Thermo Fisher Scientific (100–1300 bp, twelve fragments) using the Photo- Capt MW software 10.0 (Vilber Loumart) and photographed under UV light. Total bands number, polymorphic and monomorphic bands and band lines were scored visually. The following primers parameters were measured as follows (Abass *et al*., 2017):

- **Primer Efficiency %** $= \frac{\text{Total number of amplified bands by ISSR primer}}{\text{Total number of obtained bands}} \times 100$
- **Polymorphism %**$= \frac{\text{Total number of polymorphic bands by ISSR primer}}{\text{Total number of obtained bands by the same primer}} \times 100$
- **Discrimination Power %** $= \frac{\text{Total number of polymorphic bands by ISSR primer}}{\text{Total number of polymorphic bands}} \times 100$

All amplified bands were visually scored as present (1) or absent (0) to create the binary matrix. Only clear and reproducible amplified fragments were considered for estimation the genetic similarity coefficient and distance for all pairs of treatments according to Nie and Li (1979), as follows:

- **Genetic similarity index (GSI)** $= {}^{2A}/_{(B+C)}$

where(A) is number of similar bands in both treatments, (B) and (C) are the total number of bands in the first and second treatments.

- **Genetic Distance (GD)= 1- GSI**

The similarity coefficients were used to construct a dendrogram illustrating genetic relationship using the unweighted pair group mean average (UPGMA) method according to Sneath and Sokal (1973).

Table (1). Description of ISSR molecular markers.

ISSR Identity	ISSR Sequence 5'.....3'	Length / bp	GC %
815	CTC TCT CTC TCT CTC TG	17	52.94
818	CAC ACA CAC ACA CAC AG	17	52.94
822	TCT CTC TCT CTC TCT CA	17	47.05
834	AGA GAG AGA GAG AGA G (CT) T	19	47.36
855	ACA CAC ACA CAC ACA C (CT) T	19	47.36

Genome Template Stability (GTS)

The genome template stability was measured in all treated date palm plants with heavy metals according to the equation of Atienzar *et al*. (1999):

GTS (%)= 1- $(\boldsymbol{a}|\boldsymbol{n})\times$ **100**

Where $\boldsymbol{a}$ is the average number of changes in DNA profiles and $\boldsymbol{n}$ the number of bands selected in untreated date palm DNA profiles.

Results

Protein Analysis by SDS-PAGE

Protein analysis by SDS-PAGE of date palm plants exposed to different concentrations of Cd and Pb showed that all examined treatments produced 20 protein bands in the molecular weight of 18-73 KDa (Fig. 1A; Table 2); with four protein bands in each treatment. Three protein bands were observed in all treatments with the molecular weight of 73; 58 and 44 KDa; at the high concentration of Pb (276 mg/kg) the band with the molecular weight 33 KDa was disappeared and there was a new expressed polypeptide as 18 KDa molecular weight observed. The results of genetic similarity index (Table 3) revealed that the treatments of low and high concentrations of Cd and low concentration of Pb had the highest GSI values to the untreated date palm (GSI = 1); while the increase of Pb concentration to 276 mg/kg led to decrease this value to 0.75 according to Nei and Li's index.

The dendrogram was generated according to the protein profile and genetic similarities and showed that the heavy metals treatments of date palm plants separated these treatments into two cluster; the first cluster included control; low and high Cd concentration and low Pb concentration; while the highest Pb concentration was separated in one cluster with the highest genetic distance (GD= 0.30; Fig. 1B).

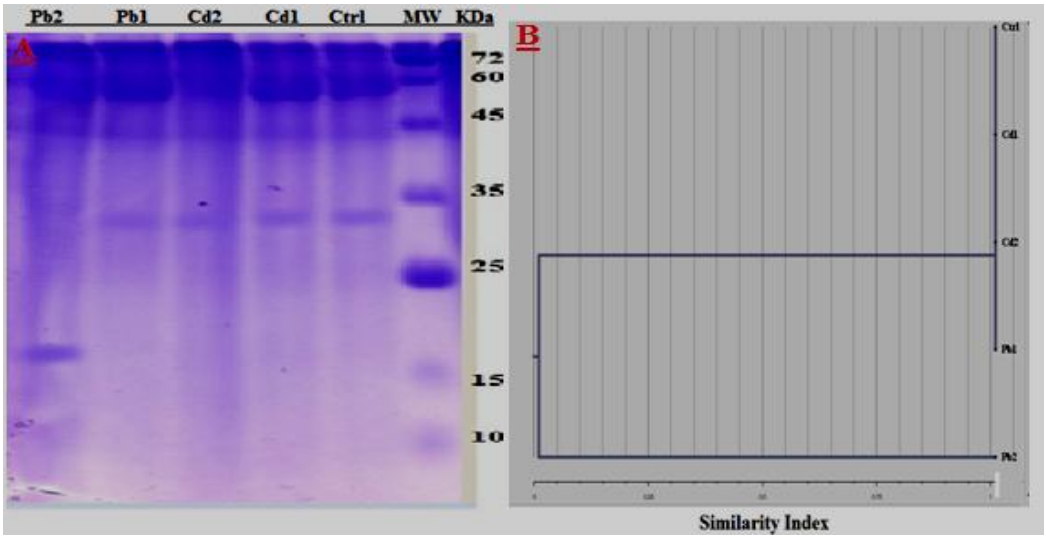

Fig. 1 A- SDS-PAGE electrophoresis of total soluble protein of date palm leaves treated with HMs.
B- A dendrogram generated by UPGMA cluster method according to protein profile.
MW: Protein molecular marker (10–225 KDa); **Ctrl**: Untreated plant; **Cd1**:3 mg/kg; **Cd2**: 9 mg/kg; **Pb1**: 100 mg/kg; **Pb2**: 276 mg/kg.

Table (2). Protein profile of treated date palm plants with different concentrations of Cd and Pb obtained by SDS-PAGE electrophoresis.

Lane number of band	Molecular weight KDa	Heavy metals treatments				
		Ctrl	Cd1	Cd2	Pb1	Pb2
1	73	1	1	1	1	1
2	58	1	1	1	1	1
3	44	1	1	1	1	1
4	33	1	1	1	1	0
5	18	0	0	0	0	1
Total number of band		4	4	4	4	4

Ctrl: Untreated plant; **Cd1**: 3 mg/kg; **Cd2**: 9 mg/kg; **Pb1**: 100 mg/kg; **Pb2**: 276 mg/kg.
1: Present of protein band. 2: Absent of protein band.

Table (3). Similarity indices according to Nei and Li's coefficients of treated date palm plants with different concentrations of Cd and Pb obtained by SDS-PAGE electrophoresis.

HMS treatments	Ctrl	Cd1	Cd2	Pb1	Pb2
Ctrl	1				
Cd1	1	1			
Cd2	1	1	1		
Pb1	1	1	1	1	
Pb2	0.75	0.75	0.75	0.75	1

Ctrl: Untreated plant; **Cd1**: 3 mg/kg; **Cd2**: 9 mg/kg; **Pb1**: 100 mg/kg; **Pb2**: 276 mg/kg.

ISSR Molecular Markers Analysis

ISSR was used as a molecular marker to evaluate the genetic stability of date palm after exposing to Cd and Pb heavy metals; five different ISSR markers were used to amplify the gDNA of date palm; four of ISSR markers (ISSR: 818; 822; 834 and 855; Fig. 2) resulted in reproducible and clear DNA bands. Generally, the total amplified bands by all examined markers was 147 bands in the molecular size of amplified bands ranged from 500 to 2200 (ISSR 822) bp; a total of 137 bands were monomorphic; while the 10 polymorphic bands were appeared in high Pb concentration treatment as shown in Table 4.

Each ISSR markers amplified between 34 (ISSR 855) to 43 (ISSR 822) bands; with an average of 36.75 bands per ISSR marker.

The highest marker efficiency was observed (29.25%) in ISSR 822, while the lowest (23.12%) was seen in ISSR 855. The highest discrimination power was reported in the ISSR marker 822 and 855 which was 30%.

The results obtained in the treatments of Cd at high and low concentration and Pb at low concentration showed identical DNA profile to the control of untreated date palm; revealing no effect on DNA integrity by these treatments. While the increasing of Pb concentration to 276 mg/kg leading to apparent variations in the DNA profile of exposed date palm; recording new appearance and disappearance of DNA bands compared with the DNA profile in control plants. This trend of results was revealed by the genetic similarity indices of Cd at high and low concentration and Pb at low concentration treatments which were identical to control plants (GSI= 1); while this value was decreased up to 0.80 in the date palm plants exposed to high Pb concentration as shown in Table 5.

The genotoxic effects of heavy metals were further evaluated using the cluster analysis in UPGMA dendrogram (Fig. 3); results revealed that Pb at high concentration was separated in one cluster with the highest genetic distance than control plants (GD= 0.20); while the treatments of Cd at high and low concentration and Pb at low concentration were grouped in one cluster with control plants revealing no toxic effects of these treatments on genetic materials of date palm.

Genome template stability of treated date palm was measured by efficient ISSR markers; and the results analysis showed that Pb at high concentration led to decrease the GTS from 100% in control plants to 70%; while Cd at high and low concentration and Pb at low concentration did not show any affect on the genetic structure of date palm as shown in Fig. 4.

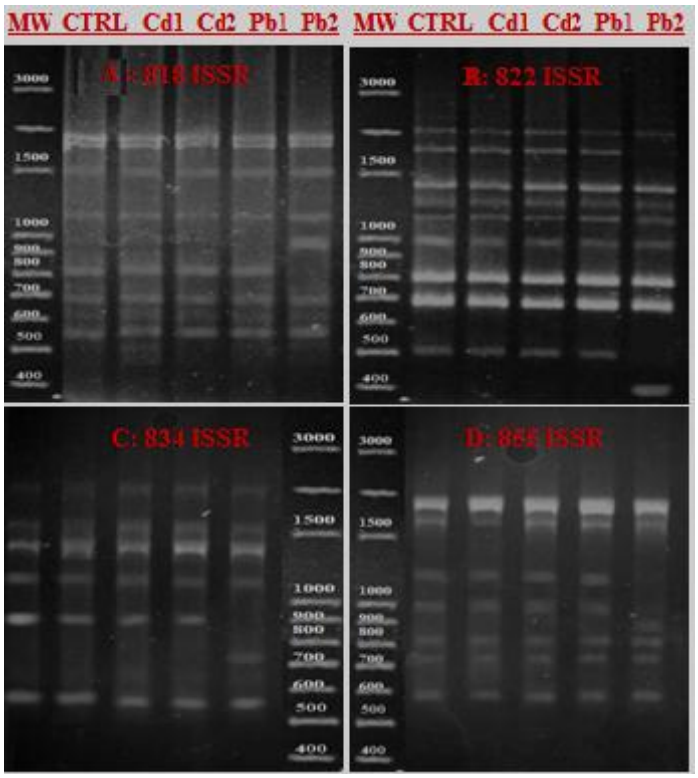

Fig. 2. ISSR molecular markers analysis of gDNA of treated date palm plants with HMs.

A: 818 ISSR; B: 822 ISSR; C: 834 ISSR; D: 855 ISSR.

MW: DNA molecular marker (100 bp); **Ctrl**: Untreated plant; **Cd1**: 3 mg/kg; **Cd2**: 9 mg/kg; **Pb1**: 100 mg/kg; **Pb2**: 276 mg/kg.

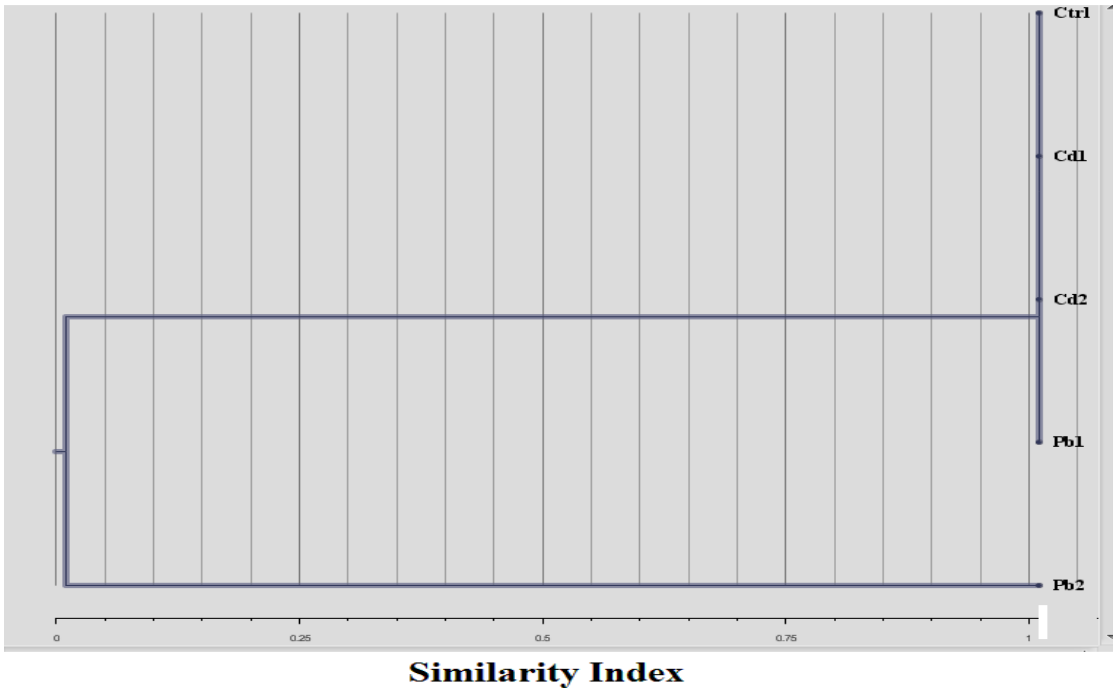

Fig. 3. A dendrogram generated by UPGMA cluster method according to ISSR molecular markers of gDNA of date palm treated with HMs.
Ctrl: Untreated plant; **Cd1**: 3 mg/kg; **Cd2**: 9 mg/kg; **Pb1**: 100 mg/kg; **Pb2**: 276 mg/kg.

Table (4). ISSR markers analysis: total number of bands, polymorphic bands, band range, primer efficiency, polymorphism and discrimination percentages.

ISSR Marker	Total No. of Bands	Polymorphic bands	Bands range bp	ISSR efficiency	Polymorphism %	Discrimination Power %
818	35	2	550-1800	23.80	5.71	20
822	43	3	500-2200	29.25	6.97	30
834	35	2	550-2000	23.80	5.71	20
855	34	3	580-1800	23.12	8.82	30
Total No.	147	10				

Table (5). Similarity indices according to Nei and Li's coefficients of treated date palm plants with different concentrations of Cd and Pb obtained by ISSR marker analysis.

HMS treatments	Ctrl	Cd1	Cd2	Pb1	Pb2
Ctrl	1				
Cd1	1	1			
Cd2	1	1	1		
Pb1	1	1	1	1	
Pb2	0.80	0.80	0.80	0.80	1

Ctrl: Untreated plant; **Cd1**: 3 mg/kg; **Cd2**: 9 mg/kg; **Pb1**: 100 mg/kg; **Pb2**: 276 mg/kg.

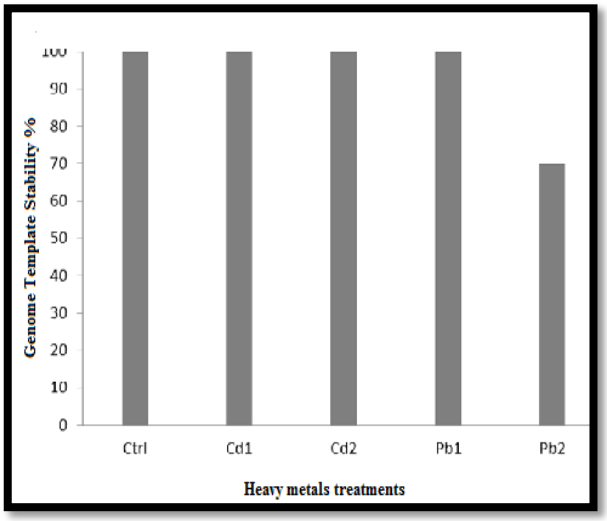

Fig. 4. Genome template stability (%) of treated date palm with heavy metals.

Ctrl: Untreated plant; **Cd1**: 3 mg/kg; **Cd2**: 9 mg/kg; **Pb1**: 100 mg/kg; **Pb2**: 276 mg/kg.

DISCUSSION

In the present study, the responses of date palm plants to Cd and Pb heavy metals treatments were evaluated using protein profile by SDS-PAGE method and ISSR molecular markers.

Protein Analysis by SDS-PAGE

Protein electrophoresis by SDS-PAGE method is one of vital technique in biology to evaluate plant responses to different stresses compared with non-stress plants (Ranjan *et al*., 2013); several studies used this technique to identify the genetic impact of different chemicals including plant growth regulators; hydrogen peroxide and sodium salt (Kairong *et al*., 2002; Ishizaka *et al*., 2002; Abbas *et al*., 2015, Abass *et al*., 2017).

Our results showed that the Cd at low and high concentrations as well as Pb at low concentration did not induce any change in protein patterns compared with control ones; while the high concentration of Pb (276 mg/ kg) led to induce the change in protein patterns (disappearance and new appearance of protein bands) compared with non treated plants. The changes in protein patterns indicate the toxicity of Pb at high concentration, previously, several literatures revealed that the Pb toxicity is dependent on its concentration (Appenroth, 2010; Abass *et al*., 2015; 2016). The loss of polypeptides bands at high concentrations of Pb could be attributed to the protein degradation which caused by the induction of ROS production in stressed plants (Shahid *et al*., 2014), or to the interaction with protein refolding process by replacing the necessarily elements such as zinc and inhibiting protein refolding and activating proteolysis as consequences (Tamas *et al*., 2014).

Regarding to the new appearance of protein band with low molecular weight (18 KDa) which induced at high Pb concentration ; thus could be a response to HMs stress; several literatures revealed that the HMs stressed plants tend to accumulate low molecular weight proteins which known as phytochelatin group and these proteins are rich in Thiol, and the second group of induced proteins are metallothioneins and these proteins are rich in cysteine amino acid; both protein groups are related to detoxification of HMs in plant (Prasad, 1995; Zagorchev *et al*., 2013; Emamverdian *et al*., 2015).

No previous studies showed the toxicity of Cd and Pb HMs on date palm using protein profiles; but there are several studies revealed the changes in protein patterns in exposed plants to HMs including *Lepidium sativum*; *Brassica* spp. and *Abutilon indicum* (Bafeel, 2010; Iqbal *et al*., 2012; Sahoo *et al*., 2015).

ISSR Molecular Markers Analysis

Our results showed the efficiency and productivity of ISSR molecular marker in detection of the genotoxicity of high Pb concentration (276 mg/ kg) on the whole date palm plants under controlled conditions. To the best of our knowledge this is the first study in revealing the toxicity of Pb on date palm using ISSR technique.

ISSR molecular marker has been used as a tool to evaluate the genetic diversity among different cultivars of date palm (Karim *et al*., 2010)

ISSR analysis showed changes in DNA patterns which were evident by the new appearance and disappearance of bands as a consequence to the treatment with high concentration of Pb compared to non-treated plants; the loss of normal DNA bands in HMs stressed plants could be attributed to the damage of DNA; point mutation and chromosomal rearrangement (Atienzar *et al*., 2000). The new DNA bands amplifications may be associated to the events such as modification of primer binding sites caused by the genotoxic affects of Pb at high concentration and induction of mutations or large deletion/ addition (Ahmed *et al*., 2012; Malar *et al*., 2014). Additionally, the changes in DNA profile could be caused by the break in DNA strand either single or double strand break (Shahid *et al*., 2014).

The genotoxic affects of Pb on date palm are in accordance with many other results revealed these affects using ISSR markers on different plants such as *Eruca sativa* and *Pistia stratiotes* (Al-Qurainy, 2010; Neeratanaphan *et al*., 2014).

The genotoxicity of Cd and Pb were further evaluated in comparison with non treated plants using cluster analysis; dendrogram revealed that Cd at low and high concentrations; as well as, Pb at low concentration did not show any damage on DNA level, all of these treatments have been clustered in one group with control plants. High Pb concentration was separated in a single group with a highest genetic distance to control plants.

Regarding the genome template stability of date palm, results showed that the treatment of Pb at high concentration (276 mg/ kg) led to decrease GTS to 70% compared to control plants, herein, our results of genotoxicity of Pb well agreed with the outcomes of several studies on various plants such as *Phaseolus vulgaris, Brassica* spp., *Sesbania grandiflora*, *Secale cereale* and *Hordeum vulgare* (Enan, 2006; Cenkci *et al*., 2010; Malar *et al*., 2014; Qzyigit *et al*., 2016; Mahfouz and Rayan, 2016).

CONCLUSIONS

The results obtained from this study highlight for the first time the genotoxic affects of heavy metals on date palm plants. Both protein profile and ISSR molecular markers seems to be good techniques in detection Cd and Pb genotoxicity. Results revealed that Cd at low and high concentrations; as well as, Pb at low concentration did not show any damage on protein and DNA levels. High Pb concentration- induced changes in protein and ISSR DNA patterns. Genome template instability has been observed in treated date palm with Pb at 276 mg/ kg.

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السمية الوراثية للكادميوم والرصاص في نخيل التمر *Phoenix dactylifera* L.

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الخلاصة

تم دراسة السمية الوراثية للمعادن الثقيلة الكادميوم (Cd) و الرصاص (Pb) في نخيل التمر *Phoenix dactylifera* L. في المختبر تحت ظروف مسيطر عليها. اذ تم تقييم السمية الوراثية باستخدام النمط البروتيني والبادئات الجزيئية ISSR. تم اختيار اثنين من تركيزات الكادميوم والرصاص هي 3 و 9 ملغم / كغم Cd , 100 و 276 ملغم / كغم Pb. أظهرت النتائج بوضوح أن الكادميوم عند التركيزين المدروسين و Pb عند التركيز المنخفض (100 ملغم / كغم) لم يحدث أي تغير في المواد الوراثية من حيث أنماط البروتين وبادئات ISSR بالمقارنة مع نباتات النخيل غير المعاملة . بينما أدى التركيز المرتفع من الرصاص (276) ملغم / كغم إلى ظهور حزمة بروتينيه جديده (18 كيلو دالتون)، بالإضافة إلى اختفاء حزمة بروتينية (33 كيلو دالتون) لوحظت في جميع المعاملات. وكشف تحليل ISSR و Dendrogram انفراد Pb عند التركيز العالي في مجموعة واحدة مع أعلى مسافة جينية (0.2 وفقا لمؤشر Nie and Li's) من معاملة السيطرة. بالإضافة إلى ذلك ، أشارت النتائج إلى أن ثبات قالب الوراثي (GTS) قد تأثر بشكل ملحوظ بالرصاص عند التركيز العالي وانخفض إلى 70٪ مما يعكس السمية الوراثية لـ Pb عند 276 ملغم / كغم.

نتائجنا تسلط الضوء للمرة الأولى على التأثيرات السمية الجينية لتركيز الرصاص في التركيز العالي على جينوم نخيل التمر.