ASSESSMENT OF LEAD STRESS USING GENOME TEMPLATE STABILITY IN *Hordeum vulgare*

HALA MAHFOUZ AND WALAA A. RAYAN

Department of Botany, Faculty of Science, Ain Shams University, Cairo, Egypt

Environmental pollution by heavy metals underwent a marked increase in the last decades due to their continuous release and accumulation in the terrestrial and aquatic ecosystems. Soil pollution by heavy metals has reasonably increased in the last decades to discharge of wastewater and waste from anthropogenic sources. Plants absorb various kinds of heavy metals when available in the soil or irrigation water (Naz *et al*., 2015). These elements easily taken up by plants and then enter food chain, resulting in a serious health issue for human including neurotoxicity, hepatotoxicity, nephrotoxicity, mutagenicity and carcinogenicity (Zhuang *et al*., 2009). The general toxic effect of heavy metals is considered to be a result of inactivation of enzymes and /or functional proteins by directly binding to them (Ginn *et al*., 2008). This may be partly due to oxidative damage by formation of reactive oxygen species (ROS) (Stohs and Bagchi, 1995; Tsuji *et al*., 2002; Valok *et al*., 2005). Emphasis on heavy metals toxicity has been directed to their abilities to induce free radical formation and biomethylation as well as alter in gene regulation (Silbergeld and Waalkes, 2000; Grover *et al*., 2010; Singh *et al*., 2010).

Among common pollutants that affect plants, lead is one of the most toxic elements because of its strong binding with organic and/or colloidal materials (Shahid *et al*., 2011). Lead used widely in many industrial processes and occurs as a contaminant in all environmental compartments; soils, water, and the atmosphere (Islam *et al*., 2007). Lead adsorption onto roots has been documented to occur in several plant species: *Vigna unguiculata* (Kopittke *et al*., 2007), *Festuca rubra* (Ginn *et al*., 2008), *Brassica juncea* (Meyers *et al*., 2008) and *Funaria hygrometrica* (Krzesłowska *et al*., 2010). Lead may enter the roots through ionic channels (Wang *et al*., 2007). Several authors have demonstrated that calcium permeable channels are the main pathway by which lead enters roots (Wang *et al*., 2007; Pourrut *et al*., 2008).

Lead (Pb) toxicity seems from its ability to bind strongly to different types of bimolecules such as amino acids, DNA and RNA, and several enzymes, thus interfering with many metabolic pathways (Naz *et al*., 2015). According to Mitler (2002), the toxicity of heavy metals including Pb and zink (Zn) may arises as a result of the generation of reactive oxygen species (ROS) that may cause wide ranging damage to proteins, nucleic acids, lipids and eventually apoptosis (cell death). The first step by which lead induces plant

Egypt. J. Genet. Cytol., 45:297-321, July, 2016
Web Site (*www.esg.net.eg*)

toxicity is the binding of the lead ions to cell membranes, producing rigidity in this component (Seregin *et al*., 2004). The second step is the disruption of microtubules and disturbances in the G2 and mitotic stages of cell division (Suradkar *et al*., 2009).

Germination is strongly inhibited by very low concentrations of Pb (Tomulescu *et al*., 2004; Islam *et al*., 2007). Inhibition of germination may result from the interference of lead with protease and amylase enzymes (Sengar *et al.,* 2009). Plant biomass can also be restricted by high doses of lead exposure (Singh *et al.*, 2010). Jiang and Liu (2010) reported mitochondrial swelling, loss of cristae, vacuolization of endoplasmic reticulum and dictyosomes, plasma membrane and nuclei injured after 48-72 h of lead exposure to *A. sativum* roots.

SDS-PAGE profiles were used to establish the mutagenic potentiality of different chemicals (Abdel Salam *et al.*, 1997a). Electrophoretic analysis of protein provides information concerning the structural genes and their regulatory systems that control the biosynthetic pathways of that protein (Khalaf *et al*., 2013). At biochemical levels, the environmental stresses cause changes in protein metabolism (Fayez, 2000).

Advantages of measuring the genotoxicity in plants at DNA level are mainly related to sensitivity and short response time. Molecular markers have advantages over morphological, cytological, and biological markers and have been applied extensively in the life sciences (Fang and Roose, 1997). They provide new tools for detection of genetic alteration in response to toxic chemicals at the level of DNA sequence and structure. ISSRs and SRAP are DNA-based markers that have become widely used in different plant research (Karaca and Izbirak, 2008; Daniel and Andrea, 2014). ISSRs permit detection of polymorphisms in inter-microsatellite loci, using a primer designed from dinucleotide or trinucleotide repeats. This method not requires information about genomic sequences and high level of polymorphism could be realized (Fernández *et al.*, 2002). SRAPs have numerous advantages such as multilocus and are an effective tool for carrying out comparative genomics (Lin *et al*., 2004). In addition, SRAP markers require no complex mix of enzymes for cutting, connection, and pre-amplification compared with that needed for amplified fragment length polymorphism markers, which makes SRAP a more effective molecular marker system for genome studies (Xu and Zhao, 2009). The resulting DNA profiles may differ due to missing bands, the appearance of new bands or change in band intensities. These bands can be scored to evaluate genetic similarities or dissimilarities.

The aim of this study was to investigate the changes in the protein and DNA profiles induced by Pb using SDS-PAGE, ISSR and SRAP analysis, and to compare these changes with growth parameters such as root and shoot growth of barley *(Hordeum vulgare)* seedlings.

MATERIALS AND METHODS

Plant material and growth conditions

The barley (*Hordeum vulgar* L.) (Giza 123) grains used in this investigation were kindly supplied from Field Crops Research Institute, Agriculture Research Center (ARC), Giza, Egypt. They were soaked for 2-3 days in distilled water, and then 15 germinated grains were selected and transferred to plastic pots containing 1 kg of steam sterilized soil at 2.0 cm depth. The experiments conducted in the present study were set up in three replicates; Hogland's solution (Hogland and Armon, 1950) was supplied to plants three times per week. To stimulate heavy metal contamination in soil, barley plant groups were treated with 50, 100 and 150 mg/l lead (in the form of Lead acetate). Control plants treated with tap water. After 15 days of sowing the growth inhibition test was performed and the root and shoot lengths were measured.

Inhibitory rate (IR%) of the above indices was calculated by the following formula: IR= (1- a/n) x100. Where (a) and (n) are the average values detected in the treated sample and control, respectively.

Soluble protein analysis using SDS-PAGE

Leaf samples (0.5 g) were collected at 15 days old seedlings grown under control and lead treatments then ground in cold pestle mortar to a fine powder under liquid nitrogen and mixed with 2 ml water-soluble extraction buffer (1 M Tris HCl, pH 8.8, 0.25 M EDTA). Samples were transferred to eppendorf tubes and left in refrigerator overnight, then vortexed for 15 seconds and centrifuged at 12000 rpm at 4°C for 20 minutes. The supernatants were collected and protein concentration was estimated using Bradford's method (Bradford, 1976) by measuring absorbance at 595 nm using spectrophotometer and expressed as µg/g fresh weight.

SDS-PAGE was performed by the methods described previously by Laemmili (1970). A volume of 50 µl of protein extract was added to the same volume of buffer (10% SDS, Glycerol, 1 M Tris HCL, pH 8.8, 0.25 M EDTA) in eppendorf tube, and 10 µl 2-Mercaptoethanol was added to each tube and boiled in water bath for 10 min, then 10 µl bromophenol blue was added to each tube before sample loading. Protein bands were visualized by staining the gel using 0.25% Coomaisse brilliant blue (R-250) and after bands becomes clear the gels were photographed and scored depends on the presence (1) and absence (0) of bands. Molecular weights of different bands were calibrated with Sigma wide range molecular marker (116-13 kDa).

Genomic DNA isolation and PCR analysis

DNA extraction: DNA was extracted from the young leaves (15 days old seedling) using the procedure reported by Doyle and Doyle (1990). DNA concentrations of total genomic DNA in each sample were estimated using a spectropho-

tometer (TU 1880 Double Beam UV-VIS).

ISSR-PCR analysis: Nine ISSRs primers listed in Table (1) were used in this study. Amplification was performed in a DNA thermal cycler (Techne. Thermal cycler) using the temperature conditions as shown in Table (2). Electrophoresis of DNA samples were performed on 1.7% agarose gel and visualized with 0.5 mg/ml ethidium bromide. DNA bands were photographed under UV light. The size of each amplification product was automatically estimated using UV soft image analyzer system.

SRAP-PCR analysis: SRAP is a PCR-based marker system with two primers, a forward primer of 17 bases and a reverse primer of 18 bases. Three out of ten pair of primers was used in this study (Table 1). The forward primers consist of a core sequence of 14 bases. The first ten bases starting at the 5′ end are "filler" sequences of no specific constitution, followed by the sequence CCGG and then by three selective nucleotides at the 3′ end (Li and Quiros, 2001). Variation in these three selective nucleotides generates a set of primers sharing the same core sequence. The reverse primers consist of the same components as the forward primers with the following variations: the filler is followed by AATT instead of the CCGG sequence. Following the AATT sequence, three selective bases are added to the 3′ end of the primer. The only rules for construction of the forward and reverse primers are that they do not form hairpins or other secondary structures, and to have a GC content of 40-50%. Further, the filler sequences of the forward and reverse primers must be different from each other and can be 10 or 11 bases long. Amplification reaction was performed in a according to the following temperature conditions as shown in Table (3). DNA bands were photographed under UV light. The size of each amplification product was automatically estimated using UV soft image analyzer system.

Data analysis

Each variable SDS-PAGE, ISSR and SRAP bands were considered as a locus, so scored as present (1) or absent (0). Genomic template stability (GTS%) was calculated according to the following equation: GTS = (1-a/n) x100, where a is the average number of polymorphic bands detected in each treated sample and n is the number of total bands in the control. percentage of polymorphism observed in both of SDS-PAGE, ISSR and SRAP profiles was calculated included disappearance of normal band, appearance of a new band or change in band intensities in comparison with control profile. It is suggested that alternations in DNA profiles due to genotoix exposure can be regarded as changes in genomic template stability (GTS, a qualitative measure of genotoxic effects) (Liu *et al*., 2007; Abdelmigid, 2010).

Statistical analysis

The statistical analysis of the obtained data was done using the Least Sig-

nificance Difference test (LSD) at 1% and 5% levels of probability (Snedecor and Cochran, 1973).

RESULTS AND DISCUSSION

Evaluation of plant growth

In the present investigations all Pb concentrations had an inhibitory effect on shoot and root lengths and it was found dose dependant compared with control (Table 4). At high concentration of lead (150 mg/l), the root and shoot length was found 2.50 and 5.32, as compared with untreated seedling which was found 10.60 and 6.50, respectively. The results showed that root length was significantly inhibited in response to increase of lead concentrations compared to the control plantlets, whereas stem elongation was partially affected. In a previous study of barley, Ali *et al.* (2004) reported that shoots continue their growth after inhibition of root growth by several metals suggesting that metals are mainly accumulated in the barley roots. Similar results were recorded with *Allium cepa* (Liu *et al*., 1994) as well as in *Zea mays* (Jiang and Liu, 2010). Seregin *et al*., (2004) showed that lead inhibits root growth as a result of disruption of cell cycle. Inhibition of root growth is regarded as the first obvious effect of heavy metals in plants (Ali *et al*., 2004). The root length was more sensitive parameter than shoot length at every Pb concentration (Faheed, 2005; Naz *et al*., 2015). The presence of heavy metals severely effects the plant growth and development than any more others environmental stress (Mujahid *et al*., 2013). Heavy metals reduce growth rates of plants by affecting many process such as mineral and water uptake (Barcelo and Poschenrieder, 1990), inhibition of enzymes activities (Tam'as *et al*., 2006), membrane function (Hern´ and Cooke, 1997), oxidation and cross linking of proteins (Ortega-Villasante *et al*., 2005), induction of DNA damage (Gichner *et al*., 2008) and inhibition of cell division (Fusconi *et al*., 2006).

Results from multiple studies demonstrate that nutrient uptake by plants is significantly affected by the presence of lead (Chatterjee *et al*., 2004; Sharma and Dubey, 2005; Gopal and Rizvi, 2008). Photosynthesis inhibition is a well-known symptom of lead toxicity (Cenkci *et al*., 2010). Even when small amounts of lead penetrate root cell membranes; it interacts with cellular components and increases the thickness of cell walls (Krzesłowska *et al*., 2010). The presence of Pb ions causes a large accumulation of ABA in roots and aerial plant parts (Cenkci *et al.*, 2010), leading to stomatal closure (Mohan and Hosetti, 1997). According to Elzbieta and Miroslawa (2005), the foliar respiration of plants is also reduced by lead exposure, because the deposition of a cuticle layer on leaf surfaces.

Changes in protein banding pattern

SDS-PAGE analysis was used by some authors to study the effect of environmental stress on protein profiles (Shehab *et al.*, 2004; Telma *et al*., 2008). The protein profiles showed qualitative and quantitative variations among the in-

vestigated samples. These variations included: the appearance of new bands, disappearance of some bands, and changes in band intensity in comparison with that of the control plants. Similar results were obtained by other authors (Fayez, 2000; Telma *et al.*, 2008).

Table (5) and Fig. (1) demonstrate the effect of the tested heavy metal (lead) on the protein banding patterns of barley. The total number of protein bands recorded was 21 bands with molecular weights ranging from 114 to 13 KDa. Only nine polymorphic bands were recorded with 42.86% polymorphism. The most visible changes in the SDS-PAGE patterns were the appearance of new bands like the band with molecular weights of 100, 30 and 17 KDa while protein bands with molecular weight of 85, 51, 47, 28, 20 and 16 KDa. were disappeared after treatment with Pb. Band intensity was also observed for the protein bands with a molecular weight of 93, 63, 65 and 43 KDa (Table 5).

The alternations in the electrophoretic profiles of seed proteins are indicative of the ability of lead (Pb) to alter the gene expression in exposed plant. The appearance of new bands may be explained on the basis of mutational events at the regulatory system of unexpected gene(s) that activate it (EL-Nahas, 2000). Telma *et al.* (2008) found that the expression level of various genes encoding heat shock proteins increased after a short term of oxidative stress treatment. The disappearance of some bands might be to the deletion of their corresponding genes (Abdelsalam *et al*., 1997a). The appearance of some bands or disappearance of novel ones could also be explained on the basis of mutational events at the regulatory genes that either activate transcription or suppress unexpected genes, respectively (Abdelsalam *et al.*, 1997a). On the other hand, changes in band intensities may be due to the induction of gene mutation at the regulator gene level that increases the rate of transcription of a particular structure gene (Gamal El-Din *et al.*, 1988). The protein profile of bean seedlings was modified after lead exposure (Beltagi, 2005). Such modification can be correlated to the change that occurs in the transcriptome profile of several enzymes.

According to Gupta *et al.* (2009), the effect of lead on protein is unclear, although high concentrations may decrease the protein pool. This quantitative decrease in total protein content is the result of several lead effects: acute oxidative stress of reactive oxygen species (ROS) (Piotrowska *et al.*, 2009), modification in gene expression (Kovalchuk *et al*., 2005), increased ribonuclease activity (Gopal and Rizvi, 2008), protein utilization by plants for the purposes of lead detoxification, and diminution of free amino acid content. However, certain amino acids, like proline, increase under lead stress (Qureshi *et al.*, 2007). Such proteins play a major role in the tolerance of the plant to lead.

The change of ISSRs profile

ISSRs profiles showed substantial differences between control and Pb-

treated seedlings with apparent changes in the number and size of amplified DNA fragments with different primers (Tables 6-a &7 and Fig. 2). Nine primers were utilized to discriminate control from barley plants contaminated with lead. These primers were able to produce reproducible bands from control and treated barley plantlets. Polymorphism was evidenced as the presence and/or absence of DNA fragments between the samples. A total of 53 amplified bands (loci) from the nine primers were identified in the control seedlings ranging from 410-1927 bp in molecular size (primer ISSR-9 and primer ISSR-8 respectively (Tables 6 & 7 and Fig. 2). ISSR patterns generated by the lead exposed plantlets were dramatically different from those obtained using control DNA. Different polymorphic bands were detected at each concentration of lead treatment for different primers. The detected % of polymorphisms was 32.08%, 33.96% and 71.70% for 50, 100 and 150 mg/l lead treatment, respectively.

Previous studies have showed that changes in band patterns observed in DNA fingerprint analyses reflected DNA alterations from single base changes (point mutations) to complex chromosomal rearrangements (Atienzar and Jha, 2006; Ozturk *et al*., 2010). The principal events that were observed in the ISSR patterns to occur subsequent to lead exposure were: loss of normal bands, and appearance of new bands in comparison with the normal control plants. Almost, the nine primers detected more than one such alteration in a given sample. The maximum number of disappearing ISSR bands (29) was found to be at the higher concentrations (150 g/l). The bands of molecular size approximately 1476 and 1000 bp disappeared and extra band with molecular size approximately of 608 bp was appeared at all concentrations (Table 7 and Fig. 2). The disappearance of bands may be attributed to the presence of DNA photoproducts (e.g. pyrimidine dimers), which can act to block or reduce the polymerization of DNA in the PCR reaction (Donahue *et al.*, 1994; Atienzar *et al*., 2000). It is suggested that the DNA damage may be serious in the majority of cells of barley seedlings exposed to toxic chemicals. At higher lead concentrations, it seems that the extent of DNA lesion is so important that the Taq DNA polymerase is more often blocked which implies a disappearance of band (depending on the extent of DNA damage). The disappearance of PCR products mainly affected the high molecular weight bands because the odds of obtaining DNA photoproducts increase with the length of the amplified fragment (Ateinzar *et al*., 2000). On the other hand, mutations (new annealing events) can only be responsible for the appearance of new bands if they occur at the same locus in a sufficient number of cells. A minimum of 10% of mutations may be required to get new PCR product visible in agarose gel to be amplified by PCR (Ateinzar *et al*., 2000). Thus, the new bands could be attributed to mutations while the disappeared bands could be attributed to DNA damage.

Among molecular markers, ISSRs represent an easy and widely adopted sys-

tem, since their use does not require any prior information about target sequences and their efficiency and reproducibility are ensured (Fang and Roose, 1997; Bornet and Branchard, 2001; Pradeep-Reddy *et al.*, 2002).

The change of SRAP profile

In the present study, SRAP was used to find marker related to lead stress and to assess genetic variation of barley under lead stress. Sequence-related amplified polymorphism (SRAP) markers have been developed, which were used to amplify coding regions of DNA with primers targeting open reading frames. The SRAP system is easy to perform gives stable and moderate yields. Moreover, SRAPs have numerous advantages such as multilocus and is an effective tool for carrying out comparative genomics (Lin *et al*., 2004). In addition, SRAP markers require no complex mix of enzymes for cutting, connection, and pre-amplification compared with that needed for amplified fragment length polymorphism markers, which makes SRAP a more effective molecular marker system for genome studies (Xu and Zhao, 2009). This method provides an alternative choice to other system for obtaining highly reproducible marker. The disappearing of a normal band and/or appearing of a new band or change in band intensities are the obvious changes in the SRAP patterns generated by Pb treatments. A total of 17 amplification products (loci) from the 3 combinations primers were identified in the control seedlings ranging from 127-1883 bp in molecular size (Tables 6-b &7 and Fig. 3).

Different polymorphic bands were detected at each concentration of lead for different primers. The detected % of polymorphisms 52.94%, 58.82% and 70.59% for 50, 100 and 150 mg/l lead treatment, respectively. The number of disappearing SRAP bands was the highest (7) in Pb treated seedlings 150 mg/l. Moreover, the number of appearing SRAP bands was the highest (4) in Pb treated seedlings 100 and 50 mg/l.

Genetic relationship among the different barley treatment plants

Genetic similarity matrices among barley treatments and the control as computed according to Dice coefficient of ISSR ranged from (0.78) to (0.96) (Table 8-a and Fig. 4-a). The lowest genetic similarity (0.78) was between control and lead treatments (150 mg/l). On the other hand, the highest genetic similarity (0.96) was between 50 mg/l and 100 mg/l lead treatments. Genetic similarity matrices among barley treatments and the control as computed according to Dice coefficient of SRAP ranged from (0.66) to (0.87) (Table 8-b & Fig. 4-b). The lowest genetic similarity (0.66) was between control and treatments (150 mg/l). On the other hand, the highest genetic similarity (0.87) was between 100 mg/l and 150 mg/l lead treatments. Genetic similarity matrices among barley treatments and the control as computed according to Dice coefficient of combination data from ISSR and SRAP ranged from (0.28) to (0.93) (Table 8-c & Fig. 4-c). The lowest genetic similarity (0.28) was between control and treatments

(50 mg/l). On the other hand, the highest genetic similarity (0.93) was between control and 100 mg/l lead treatments.

In recent decades, environmental contamination by heavy metals has risen dramatically. It is known that certain heavy metals can cause DNA damage and carcinogenic effects in animals and humans, and are probably related to its mutagenic activity. On the other hand, mutations can only be responsible for the appearance of new bands. A minimum of 10% of mutations may be required to get new PCR product visible in agarose gel to be amplified by PCR (Ateinzar *et al*., 2000). Thus, the new bands could be attributed to the mutations while the disappeared bands could be attributed to DNA damage (Abdelmigid, 2009). Lead creates breaks in double strands of DNA (Rucinska *et al*., 2004; Gichner *et al*., 2008; Shahid *et al.*, 2011). Lead may enter the nucleus and bind directly to the DNA or indirectly to protein (Naz *et al*., 2015). After binding to DNA, lead disrupts DNA repair and replication mechanisms. Lead does not induce direct genotoxic effects until it becomes attached to naked DNA (Shahid *et al*., 2011). Lead can also affect replication by replacing the zinc in the Zn-finger pattern of the enzymes that intervene in DNA repair (Gastaldo *et al*., 2007). Lead is one of the most toxic environmental and industrial pollutants causing DNA damage, elevating lipid peroxidation with a long biological half-life time and represents a serious environmental pollutant for animal and plants. It influences many plants, animal and human communities. Therefore, a deeper understanding of the mechanism of Pb toxicity is important.

Genomic stability

The genomic template stability (GTS, %) values, reflecting changes in SDS-PAGE, ISSR, SRAP & root and shoot growth profiles was calculated and presented in (Table 9 and Fig. 5). It has shown considerable variation among the treated and control plantlets. In this experiment the genomic template stability was used to reflect changes in SDS, ISSR and SRAP profiles and to compare their changes with modification in traditional growth parameters (root and shoot length) in barley plantlets. Following exposure to ascending Pb concentration, both root and shoot growth of exposed plants decreased gradually according to control group. However, shoot length was less sensitive in comparison to the two parameters. Inhibition of root growth is obvious effect of heavy metals in plants (Ali *et al*., 2004).

Genomic template stability is related to the level of DNA damage, the efficiency of DNA repair and replication. These markers would be widely applicable to study the effect of contaminants on population genetics and its adaptation to different stresses. DNA polymorphism detected using ISSR and SRAP analysis due to induced or disappearance of bands in different treatments as compared with control could be used as an investigation tool for environmental toxicology and as a

useful assay. Changes in ISSR and SRAP profile induced by pollutants can be regarded as changes in genomic DNA template stability and this genotoxic effects can be directly compared with alteration in other parameters. DNA analysis have been reported as more sensitive test than classic genotoxic tests since it was capable of detecting temporary DNA changes that may not finally manifest themselves as mutations (Labra *et al.*, 2003).

Pourrut *et al.* (2008) showed that, excessive lead accumulation in plant tissue impairs various morphological, physiological, and biochemical functions in plants, either directly or indirectly, and induces a range of deleterious effects. It causes phytotoxicity by changing cell membrane permeability, by reacting with active groups of different enzymes involved in plant metabolism and by reacting with the phosphate groups of ADP or ATP, and by replacing essential ions. Lead toxicity causes inhibition of ATP production, lipid peroxidation, and DNA damage by over production of ROS. In addition, lead strongly inhibits seed germination, root elongation, seedling development, plant growth, transpiration, chlorophyll production, water and protein content. The negative effects that lead has on plant vegetative growth mainly result from the following factors: distortion of chloroplast ultrastructure, obstructed electron transport, inhibition of Calvin cycle enzymes, impaired uptake of essential elements, such as Mg and Fe, and induced deficiency of CO_2 resulting from stomata closure.

The conclusions of the research conducted in the present study are: 1) SDS-PAGE, ISSR and SRAP analysis are a highly sensitive methods for the detection of protein and DNA damage induced by environmental pollutants like Pb 2) the growth parameters most sensitive to Pb exposure is root growth 3) increasing the concentration of Pb exposure affects the genomic template stability and may induce a gain or loss in the number of bands and 4) the genomic template stability is a highly sensitive parameter compared with the traditional methods such as root and shoot growth lengths. This study suggest that the SDS-PAGE, ISSR and SRAP analysis used in conjunction with other growth parameters can be consider a powerful tool for identifying DNA damage induced by Pb contamination, which may be useful for risk assessment of environmental contamination.

SUMMARY

Assement of genotoxins-induced DNA damage at molecular level is important in eco-genotoxicology. In this research, ISSR and SRAP were used to detect DNA damage in barley (*Hordeum vulgare* L.) seeding exposed to toxic ascending Pb at concentration of 50,100, and 150 mg/l for 15 days. Substantial inhibition of root growth was observed with an increase in the Pb concentration, whereas shoot growth was non significantly inhibited compared to the unexposed plantlets. The alternations in the SDS-PAGE of seed proteins are indicative of the ability of lead (Pb) to alter the gene

expression in exposed plant. For the ISSR analyses, 9 ISSR primers were found to produce a total of 53 amplification products (loci) from the nine primers were identified in the control seedlings ranging from 410-1927 bp in molecular size (primer ISSR-9 and primer ISSR-8 respectively). The detected % of polymorphisms was 32.08%, 33.96% and 71.70% for 50, 100 and 150 mg/l lead treatment, respectively. While for the SRAP analyses, three ISSR primers were found to produce a total of 17 amplification products (loci) from the three combinations primers were identified in the control seedlings ranging from 127-1883 bp in molecular size. Different polymorphic bands were detected at each concentration of lead for different primers. The detected % of polymorphisms 52.94%, 58.82% and 70.59% for 50, 100 and 150 mg/l lead treatment, respectively. The number of disappearing SRAP bands was the highest (7) in Pb treated seedlings 150 mg/l. Moreover, the number of appearing SRAP bands was the highest (4) in Pb treated seedlings 100 and 50 mg/l.

Results produced from SDS-PAGE, ISSR and SRAP analysis indicated that the evident changes of exposed barley seedlings included gain or loss of bands compared with the control seedlings. The polymorphisms detected by both of SDS-PAGE, ISSR and SRAP profiles can be applied as a tool in risk assessment of Pb stress on plants. The results suggested that genomic template stability (GTS) reflecting changes in SDS-PAGE, ISSR and SRAP profiles was the most sensitive endpoint compared with the traditional indices such as root and shoot growth.

ACKNOWLEDGMENTS

The authors express their deepest thanks and gratitude to Dr Hoda Barakat, Professor of Genetics, Faculty of Science, Ain Shams University for her encouragement and continuous help throughout this work.

REFERENCES

Abdel Salam, A. Z. E., A. Soliman and H. Z. Hassan (1997a). The mutagenic potentialities of two organo phosphorus compound using different biological systems, Egypt. J. Genet. Cytol., 26: 105-120.

Abdel Salam, A. Z. E., H. Z. Hassan, A. Soliman and A. Bahieldin (1997b). Differential mutagenic activities of two aromatic compounds due to different side chain as revealed by cytological analysis and biochemical genetic indices. Egypt. J. Genet. Cytol., 26: 121-142.

Abdelmigid, H. M. (2009). Risk assessment of food coloring agents on DNA damage using RAPD markers. Journal of Applied Biological Sciences, 3:116.

Abdelmigid, H. M. (2010). Qualitative assessment of cadmium stress using genome template stability in *Hordeum vulgare*. Egypt. J. Genet. Cytol., 39: 291-303.

Ali, N. A., M. Ater, G. I. Sunahara and P. Y. Robido (2004). Phytotoxicity and bioaccumulation of copper chromium using barley (*Hordeum Vulgare* L.) in spiked artificial and natural forest soils. Ecotoxicology and Environmental Safety, 57: 363-374.

Atienzar, F. A., B. Cordi, M. B. Donkin, A. J. Evenden, A. N. Jha and M. H. Depledge (2000). Comparison of ultraviolet-induced genotoxicity detected by random amplified polymorphic DNA with chlorophyll fluorescence and growth in a marine macroalgae, Palmaria palmata, Aquatic Toxicology, 50: 1-12.

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

Barcelo, J. and C. Poschenrieder (1990). Plant water relations as affected by heavy metal stress. J. Plant Nutri., 13: 1-37.

Beltagi, M. S. (2005). Phytotoxicity of lead (Pb) to SDS-PAGE protein profile in root nodules of faba bean (*Vicia faba* L.) plants. Pak. J. Biol. Sci., 8: 687-690.

Bornet, B. and M. Branchard (2001). Non anchored inter simple sequence repeat (ISSR) markers: Reproducible and specific tools for genome fingerprinting. Plant Mol. Biol. Rep., 19: 209-215.

Bradford, M. M. (1976). Rapid and sensitive methods for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analysis Biochemistry, 72: 248-254.

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

Chatterjee, C., B. K. Dube, P. Sinha and P. Srivastava (2004). Detrimental effects of lead phytotoxicity on growth, yield, and metabolism of rice, Commun. Soil Sci. Plant Anal., 35: 255-265.

Daniel, W. H. R. and D. W. Andrea (2014). Sequence-related amplified polymorphism (SRAP) markers: A potential resource for studies in plant molecular biology. Appl. Plant Sci., Vol. 2 (1400017), July.

Donahue, B. A., S. Yin, J. S. Taylor, D. Reines and P. C. Hanawalt (1994). Transcript cleavage by RNA polymerase II arrested by a cyclobutane pyrimidine dimer in the DNA template. Proc. Natl. Acad. Sci. USA, 91: 8502-8506.

Doyle, J. J. and L. Doyle (1990). Isolation of plant DNA from fresh tissue. Focus, 12: 13-14.

El-Nahas, A. I. (2000). Mutagenic potential of imazethapyr herbicide (pursuit) on *Vicia faba* in the presence of urea fertilizer. Pakistan J. Biol. Sci., 3: 900-905.

Elzbieta, W. and C. Miroslawa (2005). Lead-induced histological and ultrastructural changes in the leaves of soybean (*Glycine max* (L.) Merr.), Soil Sci. Plant Nutr., 51: 203-212.

Faheed, F. A. (2005). Effect of lead stress on the growth and metabolism of *Eruca sativa* seedling, Acta Agron. Hungarica, 319-327.

Fang, D. Q. and M. L. Roose (1997). Identification of closely related Citrus cultivars with inter-simple sequence repeats markers. Theor. Appl. Genet., 95: 408-417.

Fayez, K. A. (2000). Action of photosynthetic diuron herbicide on cell organelles and biochemical constituents of the leaves of two soybean cultivars. Pest. Biochem. Physiol., 66: 105-115.

Fernández, B. M. E., A. M. Figueiras and C. Benito (2002). The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin. Theor. Appl. Genet., 104: 845-851.

Fusconi, A., O. Repetto, E. Bona, N. Massa, C. Gallo, E. Dumas-Gaudot and G. Berta (2006). Effects of cadmium on meristem activity and nucleus ploidy in roots of *Pisum sativum* L. cv. Frisson seedlings. Environmental and Experimental Botany, 58: 253-260.

Gamal El-Din, A. Y., E. H. A. Hussein and M. A. Eweda (1988). Variations in chromosome number and its bearing on electrophortic protein banding pattern in *Vicia*. Bull. Fac. Agric. Cairo Univ., 39: 143-153.

Gastaldo, J., M. Viau, Z. Bencokova, A. Joubert, A. Charvet, J. Balosso and M. Foray (2007). Lead contamination results in late and slowly repairable DNA double-strand breaks and impacts upon the ATM-dependent signaling pathways, Toxicol. Lett., 173: 201-214.

Gichner, T., I. Znidar and J. Száková (2008). Evaluation of DNA damage and mutagenicity induced by lead in tobacco plants. Mutat. Res. Genet. Toxicol. Environ. Mutagen, 652: 186-190.

Ginn, B., J. S. Szymanowski and J. B. Fein (2008). Metal and proton binding onto the roots of *Fescue rubra*. Chem. Geol., 253: 130-135.

Gopal, R. and A. H. Rizvi (2008). Excess lead alters growth, metabolism and translocation of certain nutrients in radish. Chemosphere, 70: 1539-1544.

Grover, P., P. Rekhadevi, K. Danadevi, S. Vuyyuri, M. Mahboob and M. Rahman (2010). Genotoxicity evaluation in workers occupationally exposed to lead. Int. J. Hyg. Environ. Health, 213: 99-106.

Gupta, D., F. Nicoloso, M. Schetinger, L. Rossato, L. Pereira, G. Castro, S. Srivastava and R. Tripathi (2009). Antioxidant defense mechanism in hydroponically grown *Zea mays* seedlings under moderate lead stress. J. Hazard Mater, 172: 479-484.

Hern, L. E. and D. T. Cooke. (1997). Modification of the root plasma membrane lipid composition of cadmium-treated *Pisum sativum*. Journal of Experimental Botany, 48: 1375-1381.

Hoagland, D. R. and D. I. Arnon (1950). The water-culture for growing plants without soil. Calif. Agric. Exp. Stn. Circ., 347 (Rev.).

Islam, E., X. Yang, T. Li, D. Liu, X. Jin and F. Meng (2007). Effect of Pb toxicity on root morphology, physiology and ultra structure in the two ecotypes of *Elsholtzia argyi*, J. Hazard Mater., 147: 806-816.

Jiang, W. and D. Liu (2010). Pb-induced cellular defense system in the root meristematic cells of *Allium sativum* L. BMC Plant Biol., Doi: 10.1186/1471-2229-10-40.

Karaca, M. and A. Izbirak (2008). Comparative analysis of genetic diversity in Turkish durum wheat cultivars using RAPD and ISSR markers. J. Food Agric. Environ., 6: 219-225.

Khalaf, A. F., E. Deya, R.Mohamed , K. M. Asmaa and M. A. Abdelrahman (2013). Alteration in protein contents and polypeptides of peanut plants due to herbicides and salicylic acid treatments. J. Environmental Studies, 11: 27-36.

Kopittke, P. M., C. J. Asher, R. A. Kopittke and N. W. Menzies (2007). Toxic effects of Pb^{2+} on growth of cowpea (*Vigna unguiculata*). Environ. Pollut., 150: 280-287.

Kovalchuk, I., V. Titov, B. Hohn and O. Kovalchuk (2005) Transcriptome profiling reveals similarities and differences in plant responses to cadmium and lead. Mutat. Res. Fundam Mol. Mech. Mutagen, 570: 149-161.

Krzesłowska, M., M. Lenartowska, S. Samardakiewicz, H. Bilski and A. Wo´zny (2010). Lead deposited in the cell wall of *Funaria hygrometrica* protonemata is not

stable–a remobilization can occur. Environ. Pollut., 158: 325-338.

Labra, M., T. D. Fabio, F. Grassi and S. M. G. Regondi (2003). AFLP analysis as biomarker of exposure to organic and inorganic genotoxic substances in plants, Chemosphere, 52: 1183-1188.

Li, G. and C. F. Quiros (2001). Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. Theor. Appl. Genet. 103: 455-461.

Lin, Z. X., X. L Zhang and Y. C. Nie (2004). Evaluation of application of a new molecular marker SRAP on analysis of F_2 segregation population and genetic diversity in cotton. *Yi* Chuan Xue Bao, 31: 622-626.

Liu, D., W. Jiang, F. M. Zhao and C. Lu (1994). Effects of lead on root growth, cell division and nucleolus of *Allium cepa*. Environ. Pollut., 86: 1-4.

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

Meyers, D. R., G. J. Auchterlonie, R. I. Webb and B. Wood (2008). Uptake and localization of lead in the root system of *Brassica juncea*, Environ. Pollut., 153: 323-332.

Mitler, R. (2002). Oxidative stress, antioxidant and stress tolerance. Trends Plant Sci., 7: 405-410.

Mohan, B. S. and B. B. Hosetti (1997). Potential phytotoxicity of lead and cadmium to *Lemna minor* grown in sewage stabilization ponds, Environ Pollut., 98: 233-238.

Mujahid, F., B. S. Muhammad, E. Sana, A. Shafaqat, Z. Muhammad and A. H. Muhammad (2013). Morphological, physiological and biochemical responses of different plant species to Cd stress. IJCBS, 3: 53-60.

Naz, A., S. Khan, S. Muhammad, S. Khalid, S. Alam, S. Siddique, T. Ahmed and M. Scholz (2015). Toxicity and bioaccumulation of heavy metals in spinach (*Spinacia oleracea*) grown in a controlled environment, Int. J. Environ. Res. Public Health, 12: 7400-7416.

Ortega-Villasante, C., R. Rella´n A. lvarez, F. F. DelCampo., R. O. Carpena-Ruiz and L. E. Hern´andez (2005). Cellular damage induced by cadmium and mercury in *Medicago sativa*. Journal of Experimental Botany, 56: 2239-2251.

Ozturk, F., F. Duman, Z. Leblebici and R. Temizgul (2010). Arsenic accumulation and biological responses of watercress (Nasturtium officinale R. Br.) exposed to arsenite. Environm. and Experimental Botany, 69: 167-174.

Piotrowska, A., A. Bajguz, B. Godlewska-Zylkiewicz, R. Czerpak and M. Kaminska (2009). Jasmonic acid as modulator of lead toxicity in aquatic plant *Wolffia arrhiza* (*Lemnaceae*), Environ. Exp. Bot., 66: 507-513.

Pourrut, B., G. Perchet, J. Silvestre, M. Cecchi, M. Guiresse and E. Pinelli (2008). Potential role of NADPH-oxidase in early steps of lead-induced oxidative burst in *Vicia faba* roots. J. Plant Physiol., 165: 571-579.

Pradeep-Reddy, M., N. Sarla and E. A. Siddiq (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica, 128: 9-17.

Qureshi, M., M. Abdin, S. Qadir and M. Iqbal (2007). Lead-induced oxidative stress and metabolic alterations in *Cassia angustifolia* Vahl. Biol. Plantarum 51: 121-128.

Rucinska, R., R. Sobkowiak and E. A. Gwó´zd´z (2004). Genotoxicity of lead in lupin root cells as evaluated by the comet assay. Cell Mol. Biol. Lett., 9: 519-528.

Snedecor, G. W. and W. G. Cochran (1973). Statistical Methods. 6th ed., Iowa State University Press, Iowa, USA.

Sengar, R. S., M. Gautam, R. S. Sengar, R. S. Sengar, S. Garg, K. Sengar and R. Chaudhary (2009). Lead stress effects on physiobiochemical activities of higher plants, Rev. Environ. Contam. Toxicol., 196: 1-21.

Seregin, I. V., L. K. Shpigun and V. B. Ivanov (2004). Distribution and toxic effects of cadmium and lead on maize roots. Russian Journal of Plant Physiology, 51: 525-533.

Shahid, M., E. Pinelli, B. Pourrut, J. Silvestre and C. Dumat (2011). Lead-induced genotoxicity to *Vicia faba* L. roots in relation with metal cell uptake and initial speciation, Ecotoxicol. Environ. Saf., 74: 78-84.

Sharma, P. and R. S. Dubey (2005). Lead toxicity in plants, Braz. J. Plant Physiol., 17: 35-52.

Shehab, A. S., S. A. F. Tawab and M. M. Morci (2004). Stimulation of cell division and gene expression in *Vicia faba* L. using leaf powder of *Azadirachte Indica*. Egypt. J. Biotech., 17: 499-514.

Silbergeld, E. and K. Waalkes (2000). Lead as a carcinogen: experimental

evidence and mechanisms of action [In Process Citation]. Am. J. Ind. Med., 38: 316-623.

Singh, R., R. D. Tripathi, S. Dwivedi, A. Kumar, P. K. Trivedi and D. Chakrabarty (2010). Lead bioaccumulation potential of an aquatic macrophyte *Najas indica* are related to antioxidant system, Bioresour Technol., 101: 3025-3032.

Stohs, S. J. and D. Bagchi (1995). Oxidative mechanisms in the toxicity of metal ions. Free Radic. Biol. Med., 18: 321-336.

Suradkar, S. G., D. J. Ghodasara, P. Vihol, J. Patel, V. Jaiswal and K. S Prajapati (2009). Haematobiochemical alterations induced by lead acetate toxicity in Wistar rats. Vet. World., 2: 429-431.

Tam´as, L., B. Bo˘cov´a, J. Huttov´a, I. Mistr´ık and M. Oll´e (2006). Cadmium-induced inhibition of apoplastic ascorbate oxidase in barley roots. Plant Growth and Regulations, 48: 41-49.

Telma, E. S., M. L. Zanor and E. M. Valle (2008). Investigating the role of plant heat shock proteins during oxidative stress. Plant Signal Behav., 3: 856-857.

Tomulescu, I. M., E. M. Radoviciu, V. V. Merca, A. D. Tuduce (2004). Effect of copper, zinc and lead and their combinations on the germination capacity of two cereals. J. Agric. Sci., 15: 39-42.

Tsuji, N., N. Hirayanagi, M. Okada, H. Miyasaka, K. Hirata and M. H. Zenk (2002). Enhancement of tolerance to heavy metals and oxidative stress in *Dunaliella tertiolecta* by Zn-induced phytochelatin synthesis. Biochem. Bioph. Res. Co., 293: 653-659.

Valok, M., H. Morris and M. T. Cronin (2005). Metals, toxicity and oxidative stress. Curr. Med. Chem., 12: 1161-1208.

Wang, H., X. Shan, B. Wen, G. Owens, J. Fang and S. Zhang (2007). Effect of indole-3-acetic acid on lead accumulation in maize (*Zea mays* L.) seedlings and the relevant antioxidant response, Environ. Exp. Boi., 61: 246-253.

Xu, C. and B. H. Zhao (2009). The development and application of SRAP molecular markers. Life Sci. Instrum., 7: 24-27.

Zhuang, P., M. B. McBride, H. Xia, N. Li and Z. Li (2009). Health risk from heavy metals *via* consumption of food crops in the vicinity of Dabaoshan mine, South China. Sci. Total Environ., 407: 1551-1561.

Table (1): List of primers and their nucleotide sequences used to generate both of ISSR and SRAP markers in barley.

ISSR primes	Sequence of primers ('5 --3')	SRAP-combinations primers	Sequence of primers ('5 --3')
ISSR-1	'5- GAG AGA GAG AGA GAG AC3'-	1-PC2 Me1+ Em2	Me1: ('5- TGAGTCCAAACCGGATA-3') Em2: ('5-GACTGCXGTACGAATTTGC-3')
ISSR-2	5- GAG AGA GAG AGA GAG AT3'		
ISSR-3	5- CTC TCT CTC TCT CTC TG 3'-		
ISSR-4	5- AGA GAG AGA GAG AGA GGT 3'-	2-PC5 Me2+ Em1	Me2: ('5-TGAGTCCAAACCGGAGC-3') Em1: ('5-GACTGCXGTACGAATTAAT-3')
ISSR-5	5- ACA CAC ACA CAC ACA CGG 3'-		
ISSR-6	5- TAG AGA GAG AGA CGAG AGA G 3'-		
ISSR-7	5- GAC ACA CAC ACA CAC ACA C 3'-	3-PC11 Me3+ Em3	Me3: ('5- TGAGTCCAAACCGGAAT-3') Em3: ('5-GACTGCXGTACGAATTGAC-3')
ISSR-8	5- CCA CTC TCT CTC TCT CTC T 3'-		
ISSR-9	5- CCA CTC TCT CTC TCT CTC T 3'-		

Table (2): The ISSR-PCR reaction parameter.

Step	Tm	Time	Cycles
Initial denaturation	94	4 m	1
Denaturation	94	35 s	35
Annealing	40	45 s	
Extension	72	2 m	
Final extension	72	10 m	1

Table (3): The SRAP-PCR reaction parameter.

Step	Tm	Time	Cycles
initial denaturation	94	4 m	1
denaturation	94	30 s	5
annealing	35	30s	
extension	72	1m	
denaturation	94	30 s	35
annealing	50	30 s	
extension	72	1 m	
final extension	72	5 m	1

Table (4): Effects of Pb stress on root and shoot length of *Hordeum vulgare* seedllings.

Pb con. mg/l	root length (cm) ±S.E	% of root Inhibitory rate	shoot length (cm) ±S.E	% of shoot Inhibitory rate
0	10.60 ± 0.51	0.0	6.50 ± 0.21	0.0
50	8.01 ± 0.42*	24.43	5.97 ± 0.15NS	8.15
100	4.33 ± 0.43**	59.16	5.93 ± 0.32*	8.77
150	2.50 ± 0.61**	76.42	5.32 ± 0.22*	18.15
LSD 5%	1.11		0.22	
LSD 1%	1.60		0.30	

HS (**) = Highly significant change. S (*) = Significant change. NS = Non-significant change

Table (5): Effect of different concentrations of lead on protien electrophoretic profiles of *Hordeum vulgare.* (P = polymorphic band and M= monomorphic band)

Band No.	MW (KDa)	Lane 1 Control	Lane 2 50 mg/L Pb	Lane 3 100 mg/L Pb	Lane 4 150 mg/L Pb	Polymorphism
1	114	1	1	1	1	M.
2	100	0	0	1	1	P.
3	93	1	1	1	1	M.
4	85	1	0	1	1	P.
5	78	1	1	1	1	M.
6	75	1	1	1	1	M.
7	68	1	1	1	1	M.
8	63	1	1	1	1	M.
9	65	1	1	1	1	M.
10	51	1	1	0	0	P.
11	47	1	0	0	0	P.
12	43	1	1	1	1	M.
13	36	1	1	1	1	M.
14	30	0	0	1	0	P.
15	28	1	1	0	1	P.
16	23	1	1	1	1	M.
17	22	1	1	1	1	M.
18	20	1	0	0	0	P.
19	17	0	1	0	1	P.
20	16	1	0	1	0	P.
21	13	1	1	1	1	M.
Total	-------	18	15	16	16	% of polymor-phism = 50%

Table (6): Percentage of polymorphism and numbers of induced and disappeared bands generated.

(a) - ISSR primers among control and three barley treatments

Primers	Lead concentration mg/l						
	Control	50		100		150	
		a	b	a	b	a	b
ISSR-1	6	0	0	0	1	0	3
ISSR-2	5	1	2	1	1	0	5
ISSR-3	8	1	3	0	2	1	5
ISSR-4	5	0	1	0	0	0	5
ISSR-5	5	0	0	1	0	3	0
ISSR-6	5	1	1	2	4	1	5
ISSR-7	5	1	1	0	1	1	1
ISSR-8	10	2	3	3	2	3	1
ISSR-9	4	0	0	0	0	0	4
Total bands	53	6	11	7	11	9	29
a+b	---	17		18		38	
% polymorphism	---	32.08%		33.96%		71.70%	

(b)- SRAP primers among control and three barley treatments

Primers	Lead concentration mg/l						
	Control	50		100		150	
		a	b	a	b	a	b
SRAP-1	2	2	0	4	0	0	1
SRAP-2	7	0	3	1	1	0	7
SRAP-3	8	4	0	4	0	3	1
Total bands	17	6	3	9	1	3	9
a+b	---	9		10		12	
% polymorphism	---	52.94%		58.82%		70.59%	

a: indicates appearance of new bands, b: disappearance of normal bands
a + b: denote polymorphic bands.

Table (7): Molecular sizes (bp) of disappearance (-) and appearance (+) of DNA bands with all primers in Pb - treated barley plants.

Primer		50 mg/L Pb	100 mg/L Pb	150 mg/L Pb
ISSR-1	+	0	0	0
	-	0	1036	1288,1036,599
ISSR-2	+	732	732	0
	-	1481, 1000	1000	1000,912, 854, 805, 673
ISSR-3	+	996	0	996
	-	1476, 882, 779	1476, 779	1476, 1113, 882, 779, 552
ISSR-4	+	0	0	0
	-	731	0	1214, 1061,973, 731, 662
ISSR-5	+	0	562	785, 562, 514
	-	0	0	0
ISSR-6	+	608	608, 413	608
	-	638	1469, 797,660, 452	1318, 1207, 467, 452, 432
ISSR-7	+	695	0	695
	-	806	806	739
ISSR-8	+	1927,1529	1927, 1529, 910	668, 605, 587
	-	1414, 1161, 587	1161, 587	1927
ISSR-9	+	0	0	0
	-	0	0	730, 649, 580, 410
SRAP-1	+	600, 533	1217, 734, 533, 472	0
	-	0	0	1420
SRAP-2	+	0	1845	0
	-	1046, 848, 393	393	1046, 848, 552, 480, 432, 393, 220
SRAP-3	+	770, 638, 269, 127	770, 638, 269, 127	638, 269, 127
	-	0	0	375

(+) = appearance of new bands; (-) = disappearance of normal bands.

Table (8): Genetic similarity matrices among barley treatments and the control as computed according to Dice coefficient.

a: ISSR			
Similarity	Control	50 mg/L Pb	100 mg/L Pb
50 mg/L Pb	0.820		
100 mg/L Pb	0.820	*0.960*	
150 mg/L Pb	*0.780*	0.830	0.870
b: SRAP			
Similarity	Control	50 mg/L Pb	100 mg/L Pb
50 mg/L Pb	0.720		
100 mg/L Pb	0.730	0.840	
150 mg/L Pb	*0.660*	0.860	*0.870*
c: ISSR + SRAP			
Similarity	Control	50 mg/L Pb	100 mg/L Pb
50 mg/L Pb	*0.280*		
100 mg/L Pb	*0.930*	0.306	
150 mg/L Pb	0.680	0.613	0.640

Table (9): Genomic template stability and % of growth difference among control and three barley treatments.

GTS					
Analysis		Control	Pb treatment 50 mg/L	Pb treatment 100 mg/L	Pb treatment 150 mg/L
SDS-PAGE		100	72.22	66.67	66.67
ISSR		100	67.92	66.04	28.30
SRAP		100	47.06	41.18	29.41
Growth	Root	100	75.57	40.84	23.58
	Shoot	100	91.85	91.23	81.85

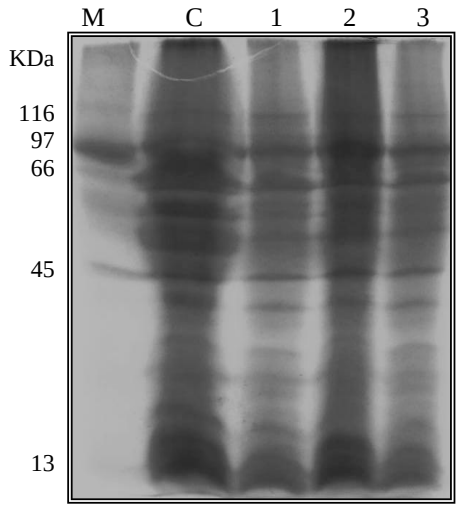

Fig. (1): Electrophotograph produced by SDS-PAGE analysis of protein patterns of *Hordeum vulgare* after treatment with different concentrations of lead.
Lane 1 =Control, Lane 2=50 mg/L Pb, lane 3 = 100 mg/L Pb and lane 4=150 mg/L Pb.

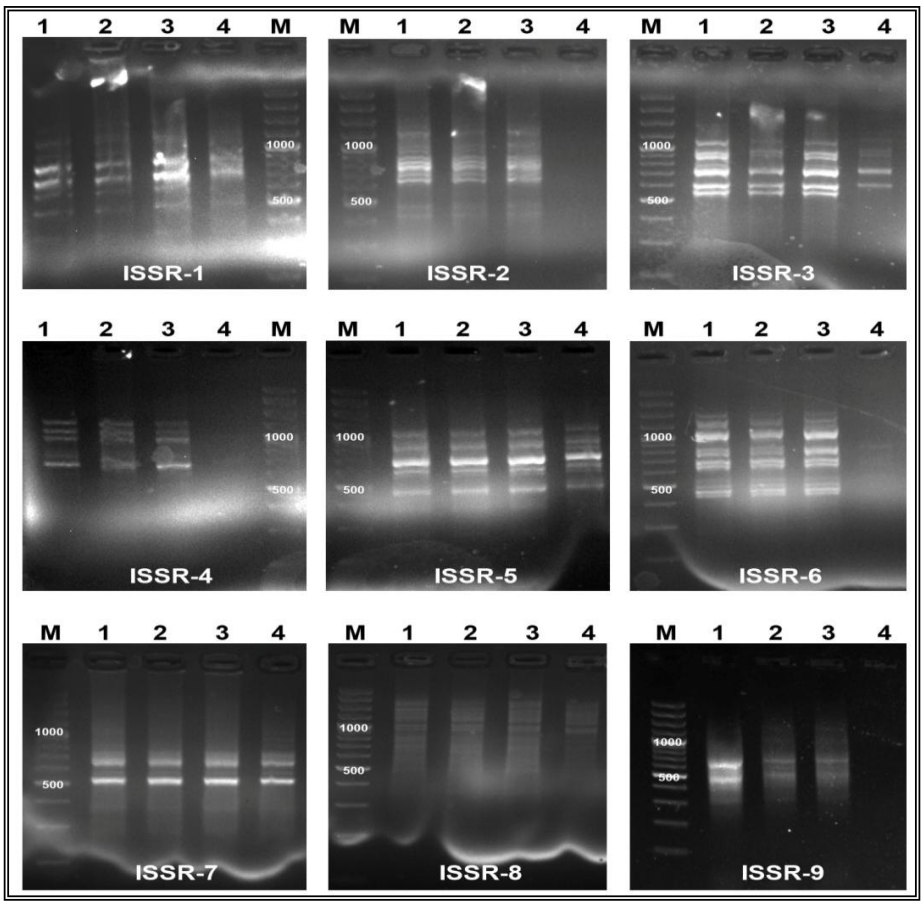

Fig. (2): The separation pattern of the ISSRs- products on 1.8% agrose gel.
Lane 1 = Control, Lane 2 = 50 mg/L Pb, lane 3 = 100 mg/L Pb and lane 4 = 150 mg/L Pb).

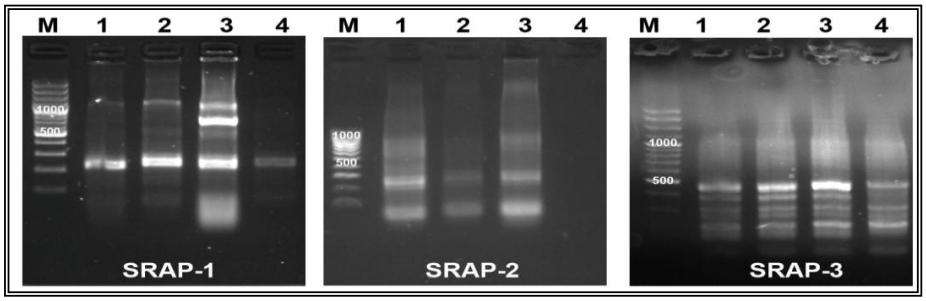

Fig. (3): The separation pattern of the SRAP- products on 1.8% agrose gel. Lane 1 = Control, Lane 2 = 50 mg/L Pb, lane 3 = 100 mg/L Pb and lane 4 = 150 mg/L Pb).

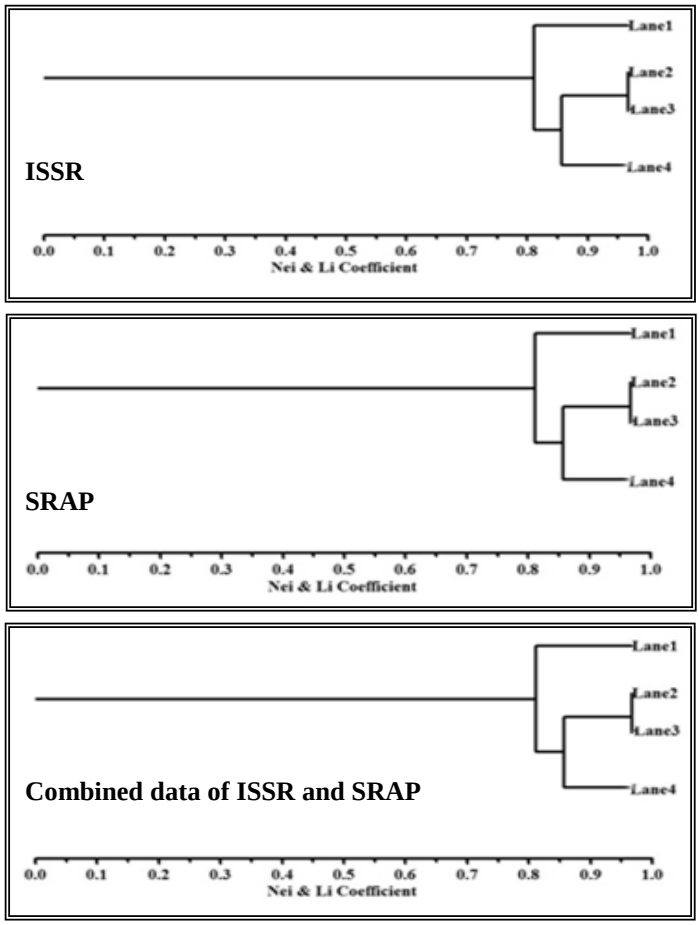

Fig. (4): Dendrogram from the three barley treatments and the control constructed data using unweighed pair group arithmetic average (UPGMA) and similarity matrices computed according to Dice coefficient. (Lane1 =Control, Lane2=50 mg/L Pb, lane 3 = 100 mg/L Pb and lane 4=150 mg/L Pb).

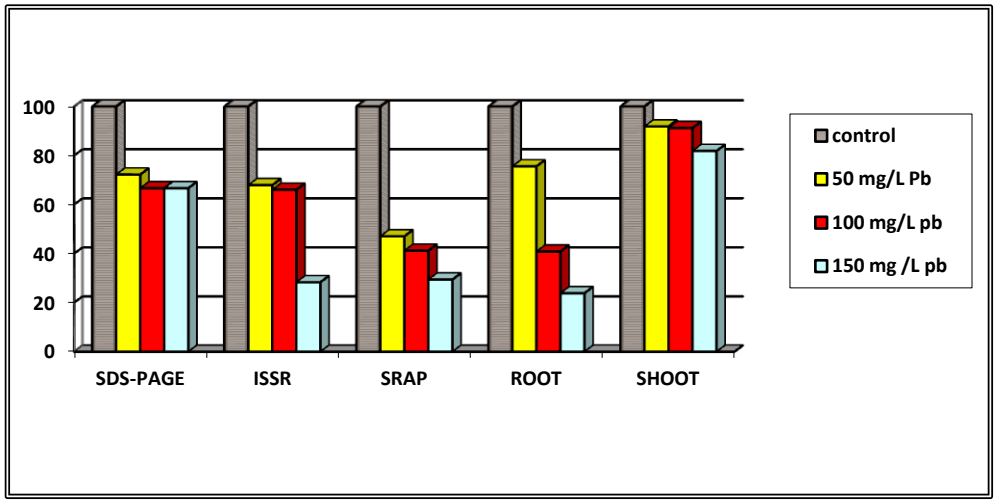

Fig. (5): Genomic template stability and % of growth difference (root and shoot) among control and three barley treatments with different concentrations of lead.