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Methylation Modelling and Epigenetic Analysis of Sunflower (*Helianthus annuus* L.) Seedlings Exposed to Cadmium Heavy Metal Stress

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ABSTRACT
Environmental pollution, especially heavy metal pollution, is an important environmental problem all over the world. Heavy metals that accumulate in high concentrations in soil and water ecosystems are known to damage most functional biomolecules such as DNA, RNA and protein in living organisms and cause genotoxicity. For example, cadmium heavy metal is one of the heavy metals that negatively affect plant growth and development. The purpose of this study was to determine the methylcytosine level in the sunflower plant genome and the changes in the methylation pattern under cadmium stress. Sunflower seeds were grown with different concentrations of cadmium heavy metal solution (Control, 20, 40, 80, 160, 320, 640 and 1280 ppm) for 3 weeks. According to the data obtained in the study, as the cadmium concentration increased, the growth and development of sunflower seedlings decreased. After detecting DNA band variations by RAPD analysis, methylcytosine levels in the sample genome were determined by CRED-RA technique. As a result of RAPD analysis, the highest GTS rate was 87.83% at 20 ppm cadmium concentration and the lowest rate was 81.75% at 320 ppm. Four different methylation patterns (Type I-IV) were determined according to the CRED-RA analysis. As a result of the study, significant changes in the DNA methylation pattern were observed by CRED-RA analysis in the sunflower genome exposed to cadmium heavy metal stress.

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Kadmiyum Ağır Metal Stresine Maruz Kalmış Ayçiçeği (*Helianthus annuus* L.) Fidelerinin Metilasyon Modellemesi ve Epigenetik Analizi

ÖZET
Çevre kirliliği, özellikle ağır metal kirliliği, tüm dünyada önemli bir çevre sorunudur. Toprak ve su ekosistemlerinde yüksek konsantrasyonlarda biriken ağır metallerin canlı organizmalardaki DNA, RNA ve protein gibi çoğu fonksiyonel biyomoleküle zarar verdiği ve genotoksisiteye neden olduğu bilinmektedir. Örneğin kadmiyum ağır metal, bitki büyümesini ve gelişmesini olumsuz etkileyen ağır metallerden biridir. Bu çalışmanın amacı, ayçiçeği bitki genomundaki metilsitozin düzeyini ve kadmiyum stresi altında metilasyon modelindeki değişiklikleri belirlemektir. Ayçiçeği tohumları, 3 hafta boyunca farklı konsantrasyonlarda kadmiyum ağır metal çözeltisi (Kontrol, 20, 40, 80, 160, 320, 640 ile 1280 ppm) ile büyütüldü. Çalışmada elde edilen verilere göre kadmiyum konsantrasyonu arttıkça ayçiçeği fidelerinin büyüme ve gelişmesi azalmıştır. RAPD analizi ile DNA bandı varyasyonları tespit edildikten sonra, numune genomundaki metilsitozin seviyeleri CRED-RA tekniği ile belirlendi. RAPD analizi sonucunda, en yüksek GTS oranı 20 ppm kadmiyum konsantrasyonunda % 87.83 ve en düşük oran 320 ppm'de % 81.75 olmuştur. CRED-RA analizine göre dört farklı metilasyon modeli (Tip I-IV) belirlendi. Çalışma sonucunda kadmiyum ağır metal stresine maruz kalan ayçiçeği genomunda CRED-RA analizi ile DNA metilasyon modelinde önemli değişiklikler gözlemlendi.

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INTRODUCTION

The sunflower plant is an oil plant that is very high commercial importance (belongs to the Helianthus annuus L. Asteraceae family) and has economic value for agriculture worldwide (Davis 1985). Sunflower (*H. annuus*) is a mostly annually grown economically valuable crop. Its seeds contain oil and are very important for nutrition. The high fatty acid rate (~70%) shows the importance of the sunflower plant. The economic value of sunflower was realized after the second world war. Subsequently, studies with physical and chemical content were carried out for quality oil with a very high oil content, good edible, refinery quality and high nutritional value. Also, Sunflower seeds are rich in potassium and vitamin-E as well as being an important food source in terms of linoleic acid (Lentz et. al., 2008; Blackmana et, al., 2011).

Important commercial oil crops such as sunflower, soybean, rapeseed, safflower, poppy, peanut and sesame are often subjected to various abiotic stresses such as drought, low temperature, salinity, excessive water, ultraviolet radiation and heavy metal contamination (Khurana and Chatterjee, 2001). Environmental pollution, especially metal pollution, is one of the global environmental and health problems affecting many organisms, from microorganisms to plants, animals and humans. Heavy metals, which can accumulate intensively in the air, water and soil have become a dangerous environmental problem that requires quick action (Yarsan et., al, 2000). Heavy metal excess in the soil causes damage to the morphological, cytological, metabolic and genomic integrity of the plants (Meyerowitz and Somerville, 1994; Hu, 2005; Kosnett, 2007; Kumar et., al, 2009; Bolukbasi and Aras, 2016; Jia et., al, 2020; Dash et., al, 2020). Heavy metals are taken into the cell by various carriers specific to their structure. They cause the formation of reactive oxygen species in organelles by affecting metabolism with various heavy metal redox reactions (Yıldız et., al, 2011; Yalcin et., al, 2020).

Some metals such as zinc (Zn), copper (Cu), manganese (Mn) and nickel (Ni) are required in low concentrations for the growth and development of plants (Kachenko and Singh, 2004). However, heavy metals such as lead (Pb), mercury (Hg) and cadmium (Cd) have severe **toxicity. Cadmium, which is not an essential element** especially for plants, is generally found in low amounts in the soil and adversely affects plant growth and development. It is not an essential nutrient for plants, but it quickly enters the cells. This metal has some serious effects on plants such as growth inhibition, decrease in enzyme activities, photosynthesis and nutrient intake (Hart et., al, 1998; Toppi and Gabrielli, 1999; Schutzendubel et., al, 2001; Kumar et., al, 2009; Bolukbasi and Aras, 2016; Jia et., al, 2020; Dash et., al, 2020).

Some plants that are called hyperaccumulators can accumulate 50 to 500 times more metal in their above and underground parts than the metal concentration in the soil (Memon et., al, 2001; Clemens, 2006). Sunflower (*Helianthus annuus* L.) which is among the plant specimens (as *Nicotiana tabacum* L., *Brassica juncea* L. and *Zea mays* L.) that can accumulate moderate heavy metals but generate high amounts of biomass, is also defined as a hyperalumulatory plant (Ozay and Mammadov, 2013; Bolukbasi and Aras, 2016; Kayakoku and Dodru, 2020).

Epigenetics is the branch of molecular biology that studies gene expression changes that are not caused by changes in DNA sequences, but are also inherited and can be passed down from generation to generation. In other words, it examines the inherited phenotypic variations that occur with non-genetic environmental effects. Such changes in DNA sequences can directly affect the cell or the organism, but there is no change in the DNA sequence (Martin and Zhang, 2007; Niu et., al, 2020).

DNA methylation is one of the best known and applied DNA modification models, which is formed by enzymatic attachment of a methyl group to the 5th carbon of cytosine (the transfer of a methyl group from S-adenosyl methionine to the 5th position of the DNA cytosine residue catalyze by DNA Methyltransferase enzymes) and plays an important role in controlling gene expression in plants. And also DNA methylation is thought to also contribute to biological defense in plants (Boyko and Kovalchuk, 2008; Pontvianne et., al, 2010; Taspinar et., al, 2017; Arslan, 2019; Shams et., al, 2020).

Many plants adapt to different stresses, such as heavy metal stress that occurs by changing their own DNA through the DNA methylation process. DNA methylation is an inherited modification that is passed down from generation to generation. The ability to remove the methyl group to return to the original DNA structure is a reversible process. Therefore, DNA methylation is an inherited mechanism and an important and common treatment method in determining the methylation pattern (Suzuki and

Bird, 2008; Chinnusamy and Zhu, 2009; Mirouze and Paszkowski, 2011; Yagci et., al, 2019; Aydin et., al, 2021).

There are many molecular analyzes used in determining methylation patterns in the genome (Cai et., al, 1996; Leljak et., al, 2004). The technique of coupled/combined restriction enzyme digestion-random amplification (CRED-RA) is an old however an effective and valid technique for detecting methylation patterns in plants. There are many studies in which the CRED-RA technique has been used successfully to determine DNA methylation patterns (Grigg and Clark, 1994; Rein et., al, 1998; Tani et., al, 2005; Karan et., al, 2012; Bolukbasi and Aras, 2016; Taspinar et., al, 2017; Arslan, 2019; Shams et., al, 2020; Aydin et., al, 2021).

In this current study, sunflowers (*Helianthus annuus* L.) groups exposed to stress with various cadmium solutions compared with control group plants for possible methylation differences were evaluated. Thus, it was aimed PCR-based CRED-RA technique was used to detect changes in DNA methylation pattern originating from cadmium heavy metal.

MATERIALS and METHODS

Growth of plant samples and cadmium stress treatments

Before planting sunflower seeds, their surfaces were sterilized with 70% alcohol and 30% sodium hypochlorite solution. The seeds were then washed three or four times with distilled water. For the germination and growth of sunflower seeds, viols prepared using sterile perlite were arranged. The viols prepared were divided into eight groups. Seven groups for different cadmium solutions and one group for the control group. Control group seedlings were irrigated only with 15 ml distilled water. The other batches were treated at a reaction volume of 15ml each for concentrations of 20, 40, 80, 160, 320, 640, 1280 ppm cadmium solution, respectively. The cultivation process adjusted in this way was continued for 21 days. At the end of 21 days, control group and plant samples treated with cadmium solution were harvested and stored at -20 degrees until DNA isolation.

DNA isolation from samples

Root fragments (200 mg) taken from samples which exposed to cadmium stress were powdered using liquid nitrogen. Subsequently, DNA was isolated from these samples. For DNA extracting, Lefort's (Lefort et., al, 1998) DNA isolation protocol was followed. Quantity and quality measurement of isolated genomic DNAs were determined by Nanodrop (NanoDrop ND-1000 Spectrophotometer, Thermo Scientific). And then it was confirmed by 1.5% agarose (containing 0.05µl/ml EtBr) gel electrophoresis.

PCR (RAPD) Procedure

The RAPD-PCR prosedure was carried out with a total reaction volume of 25 µl for each DNA samples. Amplification conditions were optimized with 200 ng of genomic DNA, 1 × reaction buffer, 3.5 mM $MgCl_2$, 20 µM dNTPs, 0.2 mM primer and 0.7U Taq DNA polymerase (Promega) and these amounts were used for PCR mix. Fourteen primers were used for RAPD-PCR reactions (Table-1). The PCR programme performed an initial denaturation step of 7.5 minutes at 95 °C, followed by 94 °C for 90 seconds denaturation, 36 °C for 60 seconds annealing and 72 °C for extension at 120 seconds. And then, the prosedure followed by at 72 ° C a final extension period of 5 minutes. The negative control was run on each of the samples to test for other types of contamination without any DNA template.

Table 1. Nucleotide sequences of primers were used for RAPD-PCR reactions
Çizelge1.RAPD-PCR reaksiyonda kullanılan primerlerin sekansları

Primers	*Nucleotide sequence (5´→ 3´)*
OPC-01*	TTCGAGCCAG
OPC-02*	GTGAGGCGTC
OPC-04*	CCGCATCTAC
OPC-06*	GAACGGACTC
OPC-07	GTCCCGACGA
OPC-08*	TGGACCGGTG
OPC-09	CTCACCGTCC
OPC-10	TGTCTGGGTG
OPC-11*	AAAGCTGCGG
OPA-08	GTGACGTAGG
OPB-07	GGTGACGCAG
OPF-05	CCGAATTCCC

*** refer to primers which used for CRED-RA analysis**

Technique of CRED-RA

DNA digestion with the restriction enzymes

MspI and HpaII enzymes were used to detect variation in methylation models of genom of samples between control and experimental groups. The CRED-RA run was carried out with a total volume of 20 µl for all samples. Approximately 1 µg of genomic DNA, 2 µl of 10X reaction buffer and 10U enzyme for restriction were used in a reaction volume of 20 µl. Microcentrifuge tubes containing the above components were kept in a 37 ° C water bath for 3 hours. Following a 3 hour incubation, samples were kept in a 95 ° C heat block for 15 minutes to inactivate the reaction.

PCR components and conditions

Approximately 200 ng digestion product, 2.5 µl 10 X of reaction preservative, 20 mM dNTPs, 2.5 µl MgCl2, 0.2 mM, 0.7 U Taq polymerase for each primer were used

in 25 µl reaction volume. Six of 14 RAPD primers (indicated in Table 1, as *) were showed monomorphic band profiles in RAPD-PCR. And so these primers were used in CRED-RA assay. For optimized reactions, an initial denaturation step of 96 °C was performed for 90 seconds. Then 45 cycles of 95 °C (denaturation) for 30 seconds, 36 ° C for 60 seconds (binding), 72 °C for 120 seconds (extension) followed by a final extension period of 72 °C for 10 minutes complete with. And then samples were confirmed by 1.6 % agarose gel electrophoresis. A negative control was used for each group to determine if there was any contamination.

Analysis of CRED-RA data

The digestion reactions were carried out via enzymes of *HpaII* and *MspI* separately. The data obtained as a result of the CRED-RA technique were evaluated with reference to Table 2. The enzymes of HpaII and MspI have different digestive abilities depending on the status of cytosine in the methylation model. The methylation models were evaluated and the band profiles were scored as yes/presence (1) and no/absence (0). While performing CRED-RA analysis, the scores obtained from the bands were evaluated according to previous studies (Liu et., al, 2005; Pan et., al, 2011; Wang et., al, 2011) and 4 different methylation patterns were determined.

Table 2. Methylation types of *HpaII* and *MspI* restriction enzymes according to their digestion
Çizelge 2. HpaII ve MspI restriksiyon enzimlerinin kesim kabliyetlerine göre metilasyon türleri

Type	Methylation Patterns	*HpaII*	*MspI*	Score of Band Profile			
				x	y	z	
Type I	CCGG GGCC	digestion	digestion	-/1	+/0	+/0	Non-methylation
Type II	**C**CGG GGCC **C**CGG GGCC	digestion	undigestion	-/1	+/0	-/1	Semi-methylation
Type III	C**C**GG GG**C**C	undigestion	digestion	-/1	-/1	+/0	Full-methylation
Type IV	**CC**GG GG**CC**	undigestion	undigestion	-/1	-/1	-/1	Full-methylation

x: PCR product is not digested by either enzyme "+" refer to digestion and "-" refer to undigestion
y: PCR product is digested by the *HpaII* enzyme
z: PCR product is digested by the MspI enzyme "1" refer to band presence and "0" refer to band absence

RESULTS

RAPD data analysis

In this study, a significant degree of polymorphism was observed in sunflower samples exposed to cadmium stress according to the results of RAPD analyzes. In 12 of the 18 RAPD-PCR primers performed in this current study, different polymorphic DNA bands were detected from the control group. It showed significant polymorphic band patterns in primers OPC 09 (57.2%), OPC 08 (55.50%), OPC 07 (50.00%) and OPC 11 (50.00%) (Table 3).

According to the GTS rates adapted with RAPD profiles, the highest rate was 87.83% at 20 ppm Cd concentration. The lowest rate was 81.75% at 320 ppm Cd stress (Table 4).

CRED-RA analysis

As a result of the analysis; it has been observed that heavy metals are effective in epigenetic mechanisms, especially in DNA methylation differences, and this situation provides resistance to cadmium heavy metal by forming different types of methylation in sunflower plants. By CRED-RA analysis, 4 different methylation types were obtained from sunflower seedlings exposed to heavy metal stress at different concentrations of cadmium. In detecting these differences, 6 different primers giving monomorphic and clear bands were used to detect methylation differences as a result of the PCR band profiles. The

Table 3. Results of polymorphism rate of primers were used for RAPD-PCR reactions
Çizelge 3. RAPD-PCR reaksiyonunda kullanılan primerlerin polimorfizm oranınları

Primers	Polymorphism rate (%)
OPC-01*	15.4
OPC-02*	12.5
OPC-04*	33.3
OPC-06*	36.4
OPC-07	50.0
OPC-08*	55.5
OPC-09	57.2
OPC-10	33.3
OPC-11*	50.0
OPA-08	37.5
OPB-07	26.3
OPF-05	25.3

*** refer to primers which used for CRED-RA analysis**

Table 4. % change of GTS rates

Çizelge 4. GKS oranlarındaki değişim yüzdesi

Samples	GTS rate (%)
20 ppm	87.83
40 ppm	87.16
80 ppm	86.48
160 ppm	85.13
320 ppm	81.75
640 ppm	83.78
1280 ppm	82.43

sequences of the primers used for this analysis are given in Table 1. And then, Table 2 is taken as a reference for determining the methylation patterns and determining their differences. While evaluating the methylation pattern, scoring was made as yes/presence (1) and no/absence (0) in line with the information in table 2. As a result of these analysis; the percentages of different methylation types have been calculated and the highest and lowest concentration percentages of the methylation types are specifically indicated (Liu et., al 2007; 2009; Karan et., al, 2012; Bolukbasi and Aras, 2016). The formulation used in the calculation is given in Table 5 in detail. The most striking point here is; Type-IV methylation has the highest value at all cadmium concentrations.

Table 5. The average (%) rates of methylation types based on data obtained from CRED-RA analysis
Çizelge 5. CRED-RA analizinden elde edilen verilere göre metilasyon türlerinin ortalama (%) oranları

	Control	20	40	80	160	320	640	1280
Type-I (%)	3.20	***2.30***	3.40	***5.60***	3.60	3.50	3.20	4.10
Type-II (%)	2.30	2.40	2.50	5.20	***5.50***	2.80	1.60	***0.80***
Type-III (%)	6.90	***8.00***	6.20	6.20	4.40	***3.80***	7.70	7.80
Type-IV (%)	87.60	87.30	87.90	***83.00***	86.50	***89.90***	87.50	87.30
Total methylated bands ratio (%)[a]	96.80	96.70	96.60	94.40	96.40	96.50	96.80	***97.50***
Full-methylated bands ratio (%)[b]	94.50	95.30	94.10	89.20	90.90	93.70	95.20	***97.40***
Semi-methylated bands ratio (%)[c]	2.30	2.40	2.50	5.20	***5.50***	2.80	1.60	0.80

aTotal methylated bands ratio (%) = [(II+III+IV)/(I+II+III+IV)]x100
bFull-methylated bands ratio (%) = [(III+IV)/(I+II+III+IV)]x100
cSemi-methylated bands ratio (%) = [(II)/(I+II+III+IV)]x100

The (%) ratio of methylation types obtained from CRED-RA analysis in sunflower samples given in Figure 1 comparatively.

And also, the (%) ratio of methylation pattern types obtained from CRED-RA analysis in sunflower samples given in Figure 2.

Additionally, the significant correlation was observed between the total methylation pattern and non-methylation pattern in sunflower seedlings subjected to cadmium stress. The R^2 value was 0.9873 (Figure 3).

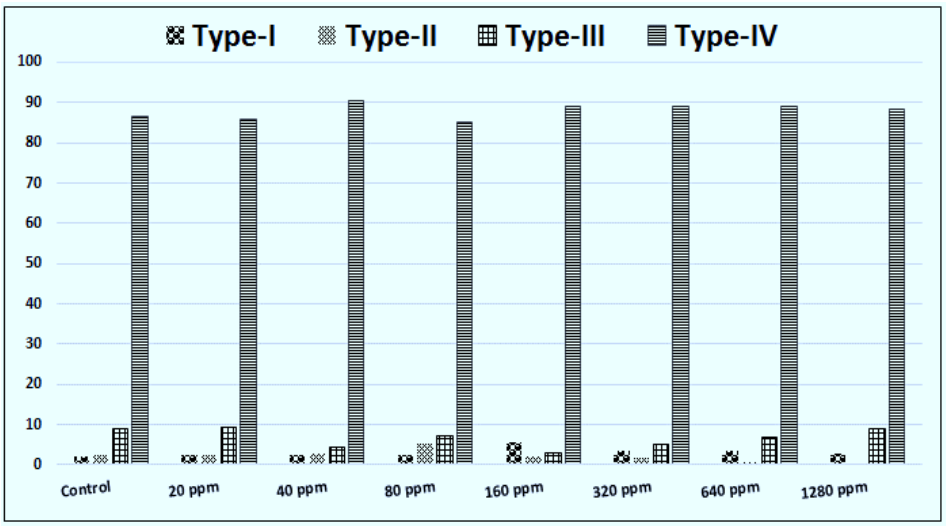

Figure 1. The average (%) rates of methylation types obtained from CRED-RA analysis in sunflower samples
Şekil 1. Ayçiçeği örneklerinde CRED-RA analizinden elde edilen metilasyon türlerinin ortalama (%) oranları

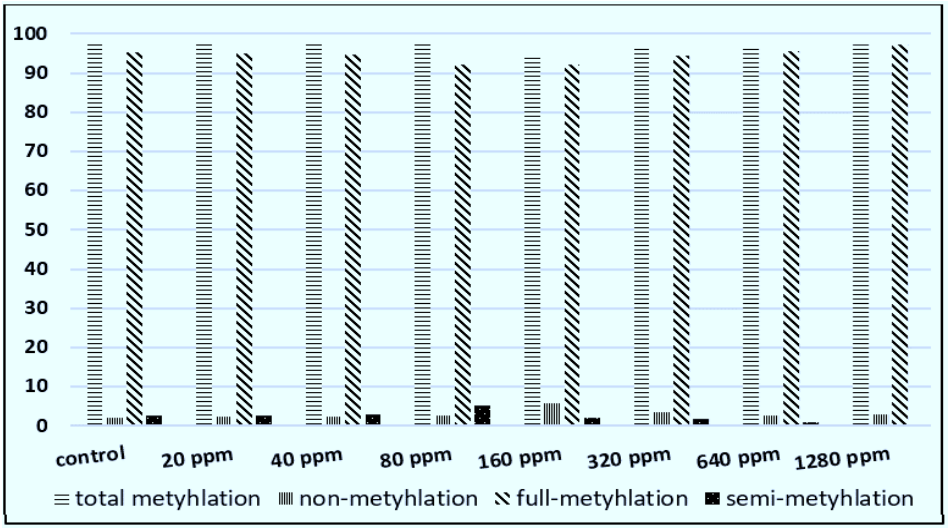

Figure 2. The (%) ratio of methylation pattern types obtained from CRED-RA analysis in sunflower samples
Şekil 2. Ayçiçeği örneklerinde CRED-RA analizinden elde edilen metilasyon modellerinin (%) oranı

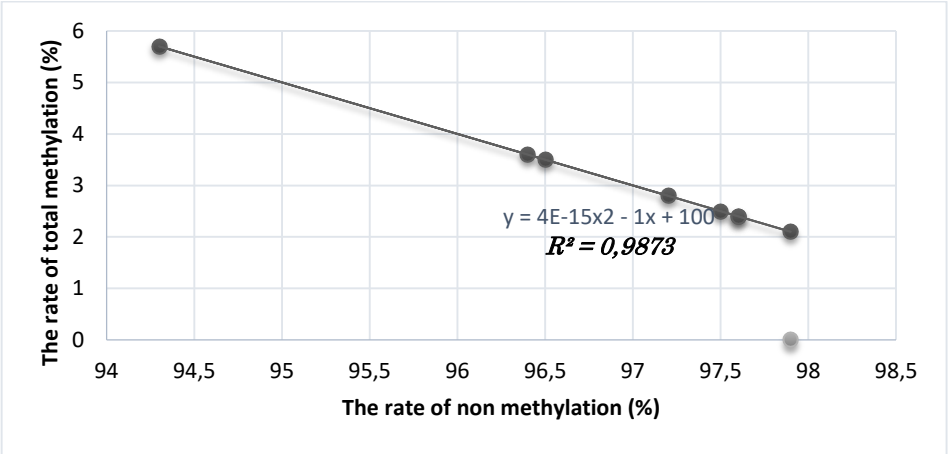

Figure 3. Influence of total methylation pattern to non-methylation pattern
Şekil 3. Toplam metilasyon modelinin (%) metillenmemiş modele (%) etkisi

DISCUSSION and CONCLUSION

One of the most important issues in environmental research is the toxic effects of heavy metals. The most important effects of heavy metals is to inhibit plant growth (Liu et., al, 2005; Pan et., al, 2011; Wang et., al, 2011; Karan et., al, 2012; Taspinar et., al, 2017; Arslan, 2019). The accumulation of heavy metals in plant tissues adversely affects the germination of seeds and the growth of roots and stems. In many studies, it has been stated that the toxicity created by heavy metals as catalysts in the oxidative degradation of biological macromolecules damages the DNA structure by causing oxidative damage (Liu et., al, 2005; 2007). It is possible to detect the toxic effects of heavy metals with molecular parameters related to DNA mutation. DNA fingerprinting techniques such as RAPD-PCR are widely used to identify DNA changes in plants induced by contaminants such as heavy metals (Theodorakis et., al, 2001; Pan et., al, 2011; Bolukbasi and Aras, 2016; Taspinar et., al, 2017; Gallo-Franco et., al, 2020; Harshitha et., al, 2020).

In this study, RAPD-PCR technique was used to detect changes in DNA band profiles. When DNA band profiles of sunflower samples exposed to cadmium heavy metal stress at different concentrations for 21 days were examined, it was seen that there were significant changes compared to the control group. RAPD-PCR profiles were analyzed by agarose gel electrophoresis containing ethidium bromide. The strips were counted one by one from the top of the strips. All RAPD-PCR profiles amplified by the primers were scored by comparison with the control group. The amplification profiles of the twelve primers were compared to the control group and the bands of the DNA fragments were scored as yes/presence (1) and no/absence (0). All primers showed significant polymorphic band patterns, particularly at OPC09 (57.2%), OPC08 (55.50%), OPC07 (50.00%) and OPC11 (50.00%). In addition, when the obtained RAPD-PCR band profiles were evaluated, the highest change in GTS ratios was 87.83% at 20 ppm Cd concentration. When RAPD-PCR data were evaluated, it was

observed that there was a significant amount of polymorphism in samples exposed to different concentrations of cadmium heavy metal stress. It was also stated in previous studies that this difference occurred as a result of mutations in the places where the primers were linked in the genome (Savva, 2000; Liu et., al, 2007; Bolukbasi and Aras 2016; Taspinar et., al, 2017; Arslan, 2019; Hosseinpour et., al, 2020). These results showed that the primers used are a strong marker or indicator for detecting mutagenic effects of cadmium heavy metal in sunflower plants. In addition, changing in GTS rates clearly highlight the importance of different cadmium stress concentrations (Conte et., al, 1998; Theodorakis et., al, 2001; Atienzar et., al, 2002; Harshitha et., al, 2020; Aydin et., al, 2021). It indicates that RAPD-PCR markers can be used successfully to detect various DNA damage in plants such as sunflower exposed to environmental pollution (Gupta and Sarin, 2009; Gallo-Franco et., al, 2020; Jia et., al, 2020).

Epigenetic mechanism like DNA methylation is an important biological defense mechanism in plants. Through DNA methylation, many plants resist various abiotic stresses such as drought, salinity, and heavy metal contamination. DNA methylation is an applied DNA modification models, which is formed by enzymatic attachment of a methyl group to the 5th carbon of cytosine (the transfer of a methyl group from S-adenosyl methionine to the 5th position of the DNA cytosine residue catalyze by DNA Methyltransferase enzymes) and plays an important role in controlling gene expression in plants (Cai et., al,1996; Leljak et., al, 2004; Suzuki and Bird, 2008; Chinnusamy and Zhu, 2009; Mirouze and Paszkowski, 2011; Bolukbasi and Aras, 2016; Arslan, 2019; Aydin et., al, 2021). Many techniques are used to detect changes in methylation patterns in the genome due to heavy metal pollution such as cadmium (Liu et., al, 2005; 2007; 2009). The CRED-RA technique used in this study is one of them (Tani et., al, 2005; Karan et., al, 2012; Bolukbasi and Aras, 2016; Taspinar et., al, 2017; Arslan, 2019; Aydin et., al, 2021).

In the study via PCR-based CRED-RA technique, based on the rates of average methylation types, the highest rate for Type-I methylation was 5.60 at 80ppm. Type-I methylation model represents non-methylated models. This indicates that methylated cytosine is not on double stranded DNA or internal methylated cytosine in a single strand. It has been regarded as unmethylated cytosine in previous studies (Liu et., al, 2007; Mirouze and Paszkowski, 2011; Karan et., al, 2012; Bolukbasi and Aras 2016; Arslan, 2019; Aydin et., al, 2021). In this regard, methylation appeared to be absent at all cadmium stress concentrations.

The highest rate at which the Type-II methylation pattern detected was 5.50% at 160ppm. Type-II methylation represents the externally methylated semi-methylation pattern of cytosine nucleotide on the DNA that is a single strand.

As stated in previous studies, the presence of inner methylated cytosine in both strands of DNA indicates Type-III or full methylation model and the presence of outer methylated cytosine indicates Type-IV methylation model. When the data were examined, it was seen that the Type-IV methylation was at the highest level in all different concentration between 20 to 1280 ppm compare to other methylation models. Additionally, the Type-III and Type-IV methylation patterns, which are full methylation types have appeared at all cadmium stress concentrations.

In conclusion, DNA polymorphisms investigated in sunflower plants in response to abiotic stress conditions were analyzed by CRED-RA technique at different concentrations of cadmium heavy metals. Significiant polymorphisms and methylation changes has been observed that changes in the level of methylation patterns have an effect on the biological defense mechanism in sunflower plants. In addition, the results of current study indicate that cadmium is a serious genotoxic agent for sunflower plants. Also this research have clearly shown that such studies will be effective in cleaning and restoring areas contaminated with various heavy metals. In addition, the hyperacumulator feature of the sunflower plant was once again determined.

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Conflicts of Interest Statement

Author had no any financial or personal relationships with other individuals or organizations that might inappropriately influence this work during the submission process.

Statement Contribution of the Author

This study's experimentation, analysis and writing, etc. all steps were made by the author.

Statement of Ethics

There is no need for an ethics committee decision for the studies in the article.

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