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Analysis of genomic stability and DNA damage in plants exposed to cement dust pollution using the RAPD analysis

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Abstract

One of the most important environmental pollutants is cement dust. In this study, RAPD technology was used to define the potential genotoxic impact of cement dust on plants. For this purpose, the DNA comparison of 12 plant species(*Convolvulus sepium, Astragalus christianus, Taraxacum androssovii Medicago varia, Alyssum murale, Artemisia spisigera, Falcaria vulgaris, Anchusa strigose, Glaucium leiocarpum, Salvia syriaca, Cryciata taurica Tragopogon albinervis)*collected from the areas of 10000 m (Control area) or 0-100 m away from Askale cement factory in Erzurum; was performed. 16 primers were used, and 390 bands were obtained. Important differences were observed in plant RAPD profiles collected from 0-100 m areas when compared to their respective controls (10000 m away from the factory). Some bands in control plants got lost, and new band formations were observed. The Genomic template stability (GTS) value decreased significantly in plants collected from 0-100 m area as compared to the controls (%56.20). Soil samples from 0-100 m and control area were collected and examined for their heavy metal contents. According to the analysis results, Nickel, Cadmium, Zinc, Lead and Copper concentrations were found to be dramatically high in the soil collected from 0-100 m as compared to the control area. These results indicate that the high heavy metal content in the soil causes the changes in RAPD profiles and GTS in plants. The data also indicated that RAPD and GTS assays are basic, effective and reproducible means of the genotoxicity control of environmental pollution.

Key words: Cement dust, Plants, Soil, Heavy Metals, Genotoxicity

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Çimento tozu kirliliğine maruz kalan bitkilerde RAPD analizi ile genomik stabilite ve DNA hasarının analizi

Özet

En önemli çevre kirleticilerinden biri çimento tozudur. Bu çalışmada, çimento tozunun bitkiler üzerindeki potansiyel genotoksik etkisini tanımlamak için RAPD teknolojisi kullanılmıştır. Bu amaçla, Erzurum'da Askale çimento fabrikasına 10000 m (Kontrol alanı) ve 0-100 m uzaklıkta toplanan 12 bitki türünün (*Convolvulus sepium, Astragalus christianus, Taraxacum androssovii Medicago varia, Alyssum murale, Artemisia spisigera, Falcaria vulgaris, Anchusa strigose, Glaucium leiocarpum, Salvia syriaca, Cryciata taurica Tragopogon albinervis*)DNA karşılaştırması yapıldı. Çalışmada 16 RAPD primeri kullanılmış ve 390 bant elde edilmiştir. 0-100m alanlardan toplanan bitki RAPD profillerinde, kontrollerine göre (fabrikadan 10000 metre uzakta) önemli farklılıklar gözlenmiştir. Kontrol bitkilerinde bazı bantların kaybolduğu ve yeni bantların oluştuğu görülmüştür. Ayrıca, GTS (Genomic template stability) değeri, 0-100 m alandan toplanan bitkilerde, kontrollerinkilerle karşılaştırıldığında önemli ölçüde azaldığı görülmüştür(%56.20). 0-100m ve kontrol alanından toprak örnekleri toplanmış ve ağır metal içerikleri bakımından incelenmiştir. Analiz sonuçlarına göre, 0-100 m'den toplanan topraklarda Nikel, Kadmiyum, Çinko, Kurşun ve Bakır konsantrasyonlarının, çarpıcı şekilde yüksek olduğu bulunmuştur. Bu sonuçlar topraktaki yüksek ağır metal içeriğinin bitkilerde RAPD profillerinde ve GTS'de değişikliklere neden olduğunu göstermektedir. Sonuçlar RAPD ve GTS testlerinin çevre

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kirliliğinin bitkiler üzerindeki genotoksik etkisini tanımlamak için temel, etkili ve tekrarlanabilir yöntemler olduğunu göstermiştir.

Anahtar kelimeler: çimento tozu, bitki, toprak, ağırmetal, genotoksisite

1. Introduction

Environmental pollutants can have deleterious effects on living organisms. The cement dust contains some toxic substances like nickel, lead, cadmium, zinc, magnesium, copper, beryllium, fluoride and sulfuric acid and its dispersal in the environment leads to some deleterious effects [1; 2; 3; 4]. Wind, rain, etc. provide the dispersion of cement dust over a wide area, and it accumulates in soil, humans, animals and plants. Cement dust pollution has been reported to have negative effects on plant development and growth. It has been reported that the toxicity mechanisms of cement dust in the organism are associated with accumulation of high heavy metal concentrations in the soil [5; 6; 7]. When rats are exposed to inhalation of cement dust, symptoms, such as focal pulmonary emphysema and atrophy of elastic fibers, have been observed in the pulmonary tissue [8]. Another study has reported the widespread prevalence of bronchitis and higher respiratory symptoms, as well as bronchial asthma, in workers exposed to cement dust when compared to unexposed individuals [9]. An increase in the risk of stomach cancer has been observed in cement factory workers. The increased prevalence of lung cancer has been observed among masons working with cement [10]. Moreover, laryngeal cancer, colorectal cancer, and colorectal tumors have been observed in workers exposed to cement dust and engaged in Portland cement production [11; 12]. The prolonged exposure to it has been expressed as a risk factor in terms of right-sided colon cancer [13]. Additionally, the mutagenic potential and genotoxic effects of cement dust have been assessed by using the analysis of chromosomal aberrations [14]. However, the effect of cement dust on DNA damage and GTS has not been elucidated in plants, and genotoxic effects of pollutants are measurable. In the present article, our aim was to identify the genotoxic impacts of cement dust pollution in different plant species, growing around a cement factory using the RAPD molecular technique.

2. Materials and methods

2.1. Plant samples

The plants utilized in the present study were collected at flowering stage in July 2013 from areas near Askale cement factory, Erzurum, Turkey. 12 wild plant species were collected at a distance of 10000 meters from the external regions of the cement factory. The above-mentioned species were identified by comparing with the voucher specimens stored in Ata Herbarium at the Department of Biology, Faculty of Science, Atatürk University, Erzurum, Turkey. The specimens of the same plant species were then collected from a distance of 0-100 meters from the external region of the cement factory. Similar-aged leaves were collected and left in thermos containing ice for later use in the experiment. The leaf specimens were rapidly transported and stored in a deepfreeze (-80^{o}C) until required for analysis. For the purpose of eliminating superficial contamination by cement dust, deionized water was used to wash all leaf specimens gathered from both sampling sites (10000 m and 0-100 m from the factory) before subjected to analysis.

Two soil samples were collected near Askale cement factory and from the control area (10000 m away from the factory) and transported to the laboratory. These samples were air dried in the laboratory. The grinding of the soil samples was done and these were sieved through a 2mm sieve. The analysis of the specimens for heavy metal contents was carried out by using “Inductive coupled plasma-optical emission spectroscopy (ICP-OES)”.

2.2. DNA extraction and RAPDs

Genomic DNA was isolated following the method described by Zeinalzadehtabrizi et al., [15] with small alterations. Standard 10-base primers provided by Operon for RAPD were utilized for screening the control (unexposed) groups. PCR reaction mix contents and PCR conditions are presented in Table 1. Screening of 40 oligonucleotide primers in total was performed, and 16 primers were chosen among them and utilized for further studies. PCR amplifications were carried out in a Bio-Rad thermocycler (Bio-Rad Laboratories Inc., Hercules, CA, USA). The reaction mixtures 30 microliter (µl) were fixed in the way described below: 100 ng of gDNA, 1× buffer (10 mM Tris–HCl, 50 mM KCl, pH=8.3), 2.5 mM of MgCl2, 10 mM of dNTP, 5 U of Taq polymerase (Cinagen Co., Iran) and 5 of mM each primer.

The PCR products were separated with agarose (1.5% w/v) gel electrophoresis at 70 V for 150 min. The nanodrop spectrophotometer (Qiagen, Qiaxpert Instrument, Germany) was utilized for checking quality and quantity of the amplified DNA products.

The RAPD bands were assessed by means of Total Lab TL120 computer software program. Genomic template stability percentage (GTS, %) was computed as described below: GTS=100-(100 x a/n), where a represents the average number of polymorphic bands determined in every specimen treated, and n represents the number of total bands in the control. RAPD profile polymorphisms were manifested as, the disappearance of a normal band and the appearance of a new band in comparison with the control. The average value was counted up for every experimental group. For the purpose of computing the polymorphism value %, the 100 x a/n formula was utilized.

Table 1. PCR reaction mix contents and conditions

PCR Reaction Mix Contents	
PCR buffer	1X
$MgCl_2$	2.5 mM
Dntp	10 mM
gDNA	100 ng
Primer	5 mM
Taq DNA polymerase	5 *U*
Total volume of each sample	30 µL
PCR Conditions	
2 min at 95°C; 2 cycles of 30 s at 95°C, 1 min annealing at 37°C, 2 min extension at 72°C; 2 cycles of 30 s at 95°C, 1 min at 35°C, 2 min at 72°C; 41 cycles of 30 s at 94°C, 1 min at 35°C, 2 min at 72°C; by a final 5-min extension at 72°C.	

3. Results

Table 2 presents the RAPD profiles of plants collected from 0-100 m and 10000 m, generated from 16 primers. Differences were determined in the banding patterns upon examining the plant RAPD profiles, which were distinctly demonstrated by the appearance/disappearance of a number of bands when comparison of plants growing around the cement factory and from control sites was performed. All RAPD profiles showed the differences. All the bands formed varied in the range of 50 bp and 2000 bp. There were polymorphic bands in every plant for primers. The polymorphism value was determined as; P (%) = 0, 37.8, 33.3, 43.7, 33.9, 25.76, 40.8, 23.02, 24.39, 22.66, 31.07, 24.34 and 21.31 for 12 wild plant species, respectively (Table 2). Polymorphism resulted from the loss and/or gain of amplified bands in the plants exposed to dust as compared to the controls under all conditions. Alterations in the RAPD patterns were recorded as decreases in GTS.

The results of the heavy metal analyses of soil samples collected from 0-100 m and 10000 m from the cement factory are presented in Table 3. The cadmium concentrations of the soil at 0-100 m and 10000 m from the factory were 0.024 ppm and 0.338 ppm respectively. The lead Copper nickel and zinc concentrations around the factory were approximately four times higher than the control soil. This situation can be attributed to the fact that the heavy metal contents of the soil around the cement factory increases due to dust em erging during cement production.

4. Conclusions and discussion

The cement industry is an important source of air pollution. Some authors have shown that the dust emerging during cement production causes negative impacts on the health of cement factory employees, plants and some animals [14; 16]. Earlier papers on the effect of cement dust indicate that it causes liver abnormalities, carcinogenesis, pulmonary disorders and affects chromosomal aberrations frequency in the cement factory workers [12; 13; 14].

In the present study, RAPD profile changes and a decrease in GTS were observed in the plants exposed to cement dust. Various polymorphic bands in RAPD band were acquired, and decrease in GTS demonstrated that cement dust is genotoxic (Table 2). A possible mechanism that can explain the effect observed in our study is the generation of reactive oxygen species (ROS). Some studies have demonstrated that exposure to cement dust causes the overproduction of ROS and suppresses the activity of enzymatic antioxidants in plant tissues, and leads to oxidative stress [3; 17; 18; 19; 20].

Deoxyribose, purine and pyrimidine bases in the DNA molecule may be attacked by ROS, which can lead to DNA strand break. As a result of this the possibility of chromosome/chromatid fragmentation may increase, and therefore RAPD band changes may take place. The heavy metal content analysis of the soil surrounding the cement factory has revealed that the concentrations of lead, cadmium, copper, nickel and zinc were generally observed low at a distance higher than 10000 m from the cement factory. Different scientists have obtained similar findings in relation to genotoxic impacts of the same heavy metals on various plants using RAPD [21; 22].

Although copper, nickle and zinc are very important for plant development and growth, high concentrations of these elements adversely affect organisms and may generate ROS in the cells. Nearly all cellular macromolecules, as well as DNA, may be damaged by free radicals [21; 23; 24]. Different genotoxic tests, including RAPD, comet, micronucleus, or chromosome aberration assays, have been employed in various studies to demonstrate the genotoxic impact of lead and cadmium on plants. Their genotoxic effects have been been explained as affecting the DNA synthesis and their functioning as an enzymatic inhibitor of the enzyme system, which is necessary for the chain reaction of DNA synthesis and lesions caused by ROS [21; 22; 23; 25;26].

In conclusion, it can be said that cement dust has a genotoxic effect and leads to DNA damage in different plant species, growing around cement factories without filters in the outlet chimneys, and this can be attributed to the presence of heavy metals in the soil surrounding the cement plant.

Table 2. Changes of GTS and polymorphism values of all used primers

Primers	Control	*Convolvulus sepium*	*Astragalus christianus*	*Taraxacum androssovii*	*Medicago varia*	*Alyssum murale*	*Artemisia spisigera*	*Falcaria vulgaris*	*Anchusa strigose*	*Glaucium leiocarpum*	*Salvia syriaca*	*Cryciata taurica*	*Tragopogon albinervis*
OPA-13 CAGCACCCAC	100	78.00	91.00	69.00	56.70	84.30	70.00	78.30	91.40	100.00	57.90	84.20	63.90
OPH-17 CACTCTCCTC	100	35.00	76.00	64.30	60.00	79.50	100.00	92.40	89.30	77.60	77.80	87.30	79.50
OPA-2 TGCCGAGCTG	100	43.00	62.80	47.90	70.00	50.70	82.00	100.00	67.50	79.40	43.90	76.10	59.30
OPA-1 CAGGCCCTTC	100	38.00	45.10	44.20	85.00	83.10	61.00	87.70	56.00	67.50	75.60	90.40	60.90
OPA-6 GGTCCCTGAC	100	75.90	31.00	79.00	100.00	55.60	72.70	91.30	100.00	91.70	71.80	75.30	84.70
OPH-14 ACCAGGTTGG	100	83.00	42.40	87.00	71.00	93.20	47.00	67.90	60.10	49.80	89.40	64.10	91.90
OPH-18 GAATCGGCCA	100	42.00	100.00	40.00	67.00	60.50	59.00	71.70	64.30	87.30	85.70	56.30	87.80
OPY-6 AAGGCTCACC	100	86.00	39.50	35.90	78.00	78.00	36.00	93.40	71.90	65.80	64.30	82.90	73.50
OPY-1 GTGGCATCTC	100	59.00	74.00	61.00	65.90	94.00	48.00	65.00	74.80	54.60	80.00	64.80	69.60
OPY-8 AGGCAGAGCA	100	51.70	57.00	74.00	47.00	83.00	64.50	72.40	84.90	92.40	76.00	78.80	70.70
OPY-15 AGTCGCCCTT	100	60.00	81.00	39.80	80.00	91.00	54.00	87.30	79.90	75.50	67.00	69.30	55.00
OPY-16 GGGCCAATGT	100	44.00	94.20	48.90	63.80	72.00	57.00	88.00	86.50	56.80	67.80	60.80	74.90
OPW-1 CTCAGTGTCC	100	69.60	100.00	50.00	75.60	68.00	33.00	74.30	83.40	84.40	74.00	91.90	78.30
OPB-8 GTCCACACGG	100	72.00	44.40	54.00	64.00	89.00	55.70	43.20	66.60	79.00	60.00	77.60	86.40
OPW-7 CTGGACGTCA	100	81.00	71.00	72.00	42.90	52.00	67.00	74.00	73.40	82.70	90.20	70.90	60.70
OPW-5 GGCGGATAAG	100	76.10	89.00	42.00	36.50	54.00	39.00	44.80	59.80	93.00	89.30	80.00	66.00
GTS %	100	62.10	66.70	56.20	66.0	74.24	59.10	76.98	75.61	77.34	68.93	75.66	72.69
Polymorphism %	0	37.80	33.30	43.70	33.90	25.76	40.80	23.02	24.39	22.66	31.07	24.34	21.31

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