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Detection of Environmental Contaminants by RAPD Method

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A B S T R A C T

RAPD technique is already extensively used for species classification, genetic mapping and phylogenetic study. In addition, their use in surveying genomic DNA for evidence of various types of DNA damage and mutation shows that RAPD-PCR may potentially constitute a biomarker assay for the detection of damage and mutational events in cell of bacteria, plants and animals. Being an effective investigation tool it is applied in genotoxicity, carcinogenesis studies and environmental toxicology research. Detection of genotoxic effects using RAPD involves the comparison of profiles generated from control and treated DNA. RAPD determines the genomic template stability which is related to level of DNA damage, so it helps in identification of various environmental pollutants with cytotoxic potential.

Introduction

Along with rapid urbanization and development of modern industry, soil polluted with heavy metals and various other contaminants has been identified in recent world. Impact assessment of contaminants in soil is important issue in environmental quality study and remediation of contaminated land. Among various heavy metals cadmium and chromium have immense uses in industries and produced in household wastes (MSW), so they have threatening impact on soil pollution. In recent years several plant species have been used as bioindicators of genotoxicity and various RAPD based (RFLP, RAPD, AFLP) tests have been developed to evaluate the toxicity of environmental contaminants (Sava, 1998). The novel application of RAPD fingerprinting technique to detect genotoxin induced DNA damage to plant from contaminated sites is well practiced today (Liu *et al*., 2009). Genotoxicity describes a deleterious action on cell's genetic material affecting its integrity. This includes chemical compounds and certain types of radiations. These substances are all those with affinity to interact with DNA. Cd induces DNA damage such as single stranded, double stranded breaks, modified bases, abasic sites, DNA protein crosslinks, oxidized bases and bulky adducts (Atesi *et al*., 2004; Liu *et al*., 2009; Cencki *et al*., 2009). As genotoxicity of Cd is directly related to its effect on structure and function of DNA, it can be assayed or determined by

a number of laboratory methods. But it is necessary to develop a reliable and reproducible genotoxicity assays which can be used in addition with traditional assays for detecting any impairment of population parameters (such as growth, reproduction and viability of offspring) due to heavy metal pollution of soil. Now RAPD has been successfully and extensively used as a sensitive and reliable method to detect Cd induced DNA damage such as mutational phenomenon, rearrangements, point mutation, small inserts or deletions of DNA and ploidy changes in cells of plant and animals and bacteria (Theodorakis *et al*., 2006; Shahratash *et al*., 2010). Some researches on DNA alterations in plants induced by soil contamination stress have been reported worldwide (Unyayar *et al*., 2006; Enan, 2006).

Use of RAPD in genotoxicity and ecotoxicological study

In recent years several plant species have been used as bioindicators and several tests have been developed to evaluate the toxicity of the environmental contaminants. Assessment of genotoxin induced DNA damage and mutation at molecular level is important in ecogenotoxicity study. In soil genotoxicity study, advances in molecular biology have led to the development of a number of selective and sensitive PCR based assay for DNA analysis. DNA alterations detected by RAPD analysis offered a useful biomarker assay for the evaluation of genotoxic effects of heavy metals in C*apsicum annum* (Aslam *et al*., 2014). Cd has the capacity not only to cause morbidity to the exposed organisms, but also has the potential to induce genotoxic effect has only established by RAPD (Cimino 2006; Theodorakis *et al*., 2006, Azimi *et al*., 2013). RAPD, one of the PCR based molecular marker techniques, is simple, rapid and low cost assay. The knowledge of genome is not required; in addition a single short random oligonucleotide primer is used. RAPD assay detects wide range of DNA damages (point mutations, inversions, deletions) and at the same time large number of samples can be studied. RAPD does not require radiolabelling for visualisation. In RAPD studies, similarities and diversities are described by appearance of new bands, disappearance of bands, and variation in band intensities. Ecotoxicological literature displayed that RAPD assay is a fundamental tool to evaluate the effects of toxicants on organisms under optimized conditions. The presence, absence and intensity of bands are related to DNA damages, mutations by genotoxicants. RAPD assay was successfully used to monitor DNA changes induced by heavy metals such as lead, cadmium, copper (Körpe and Aras, 2011), UV and x-ray (Kuroda *et al*., 1999). DNA changes include damages and mutations that can be generated by toxicants directly and/or indirectly. According to RAPD profile, the genomic template stability (GTS %) could be calculated as '100 – (100 (*a*/*n*))' where '*n*' is the number of bands in control RAPD profile and 'a' the average number of changes in sample profiles (Amina, 2012). GTS is related to the level of DNA damage, the efficiency of DNA repair and replication. DNA damages and mutations may alter a primer binding site and thus genomic template stability changes and polymorphism occurs within dose-dependent treatments and untreated organisms. Erturk *et al*., (2013) established how DNA polymorphism and genomic template stability (GTS) value was significantly affected in Cr polluted maize seedling by RAPD. DNA damage in the root tip of maize seedling under Cd stress became evident by the presence and/or absence of DNA fragment in the treated samples compared to the control group was

detected by RAPD analysis (Shahrtash *et al*., 2010). They have claimed that DNA polymorphism detected by RAPD can be considered as a useful tool, to detect environmental contaminants. In their work, Qurainy and his coworkers (2010) has reported that RAPD polymorphism can be utilized to detect genotoxicity of Cd, Pb and Zn in *Eruca sativa*. Only three decamer primers used out of twenty gave single and polymorphic bands, but other 13 primers produced 5 bands. Multiple metal genotoxicity assessment showed same remarkable result in RAPD profile of *Urtica dioica* (Gjorgieva *et al*., 2013). DNA damage was detected by RAPD in Barley seedling treated with Cd (30-120 mg/L) (Liu *et al*., 2009). Their result showed variation in band intensity, loss of normal bands and appearance of new bands compared with normal seedling. *Hydrilla verticillata* and *Ceratophyllum demersum* treated with 10µM/L Cd showed change in chlorophyll content, protein content and DNA profile and DNA damage was investigated by RAPD analysis (Gupta *et al*., 2009). In their study Cenkci and his coworkers (2009) used RAPD to detect DNA damage in roots and leaves of *Phaseolus vulgaris* exposed to Hg, B, Cr and Zn. They reported that polymorphism was evident by the appearances or disappearances of DNA bands in treated plant in comparison to the controlled ones. A study was conducted by Qari (2010) to investigate the genotoxic and antigenotoxic effect of aquous extract of *Costus speciosus* in the *Allium cepa* root tip. Through RAPD banding pattern antigenotoxic capacity of *Costus speciosus* extract was established. Enan (2006) studied the genotoxic potential of Pb, Cd, Cu and Mn on DNA integrity of kidney- bean applying RAPD technique and found distinct polymorphism between control and stressed plant. A total of 467 RAPD fragments in RAPD profiles were detected by using six random primers (decamers) and 224 of these fragments showed polymorphism. There was a distinct distance between the band patterns of treated plants and the control samples when the cluster method was applied. Genotoxic effect of different concentration of Ni and Co on maize studied by Erturk *et al*., (2012) revealed unique polymorphic band profile evident by appearances of new bands and decrease in genomic template stability with increasing concentration of metals. Soydam and his coworkers (2012) compared the effects of Cu and Zn treatments on root elongation, dry weight, total protein and changes in RAPD bands profiles of cucumber. Cumulative and antagonistic effects were observed between Cu and Zn contamination in terms of population parameter and RAPD band profiles. RAPD analysis can be applied as a suitable biomarker assay for the detection of genotoxic effects of plant allelochemicals and pharmacological products also. Racco *et al*., (2014) used RAPD successfully for molecular characterization of *Dicentrarchus labrax* embryonic cells (DLEC) as a tool to detect DNA alterations by pharmaceutical products in environmental toxicological study. Investigation of Kecek and his group (2012) revealed the genotoxic effect of the essential oils of catmint (*Nepeta meyeri*) against two weeds (*Bromus danthoniae* and *Lactuca serriola*) and two crop plants (*Brassica napus* and *Zea mays*) through RAPD. Genotoxic properties of essential oils extracted from dill (*Anethum graveoleus*) and fennel (*Foeniculum vulgare*) seeds were studied using RAPD on male rats (Alakilli, 2011). The use of Lichen as a bioindicator of environmental pollution has been established by Duman *et al*., (2011). They reported major variation in RAPD profile of *Evernia prunastri* exposed to the toxicity of polluted air smoke and waste discharge of iron steel factory. Aras

and his coworkers (2011) studied the impact of environmental pollutants and heavy metal accumulation in lichen (*Pseudovernia furfuracea*) DNA through RAPD. Bozari and Aksakal (2012) evaluated the genotoxic potential of Trifluralin, a dinitroaniline herbicide on *Zea mays* by RAPD method and reported loss of normal bands and appearances of new bands in compare to control which was dose dependent. Over the last few years there has been a noticeable increase in studies aimed at evaluating the genotoxic effects of drugs through RAPD. Racco and his group (2011) studied the genetic alteration in Zebra fish (*Danio rerio)* exposed to Cabramazepine, a known antiepileptic agent and Diclofenac, a non steroid anti inflammatory drug through both RAPD and comet assay. The amplified product from the individuals showed significant changes in their electrophoretic pattern with respect to negative control. Results of the investigation of Abdelmigid (2009) indicated the DNA polymorphism detected by RAPD analysis could be a useful biomarkers assay for the detection of genotoxic effects of food dyes also.

It is concluded from all these studies that RAPD analysis could be a useful tool for quick detection of genotoxic effect of heavy metals, air pollutant, UV radiance, pharmaceutical molecules and herbicides in plants and comparison between treated and untreated genomes is necessary to evaluate how environmental pollutants modify the structure of DNA in living organisms.

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