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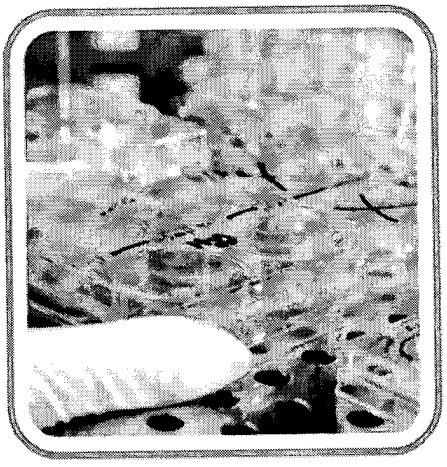

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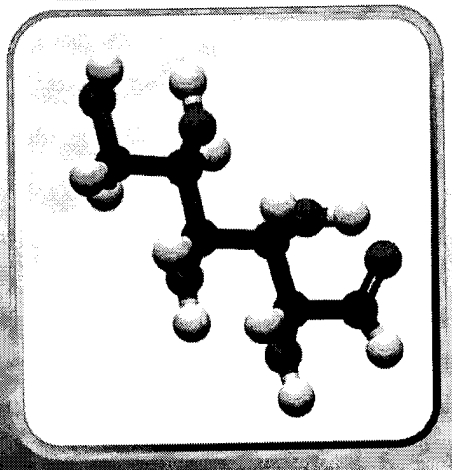

СОДРЖИНА

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елиминација на токсичните ефекти на металите и одржувањето на структурниот и метаболичкиот интегритет. Карактеристичното зголемување на продукцијата на ROS, е претпоставен механизам за оксидативниот стрес, но потребни се дополнителни истражувања на молекуларно и субклеточно ниво за подлабоко проникнување во разбирањето на точниот механизам на токсичност на металите.

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ASSESSMENT OF GENOTOXICITY OF XENOBIOTICS BY RAPD-PCR

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With fast economic development and industrialization, a vast range of genotoxic chemicals were produced, and distributed in the environment. These chemicals adversely affect living organisms, and often lead to serious diseases to human being. Due to the highly conserved structure of the genetic material, it is possible to use a broad variety of species including bacteria, yeasts, animals and plants in genotoxicity tests. Higher plants provide a useful genetic system for screening and monitoring environmental pollutants. Recently, advances in molecular biology have led to the development of a number of selective and sensitive assays for DNA analysis in eco-genotoxicology [Theodorakis and Bickham 2004]. Random amplified polymorphic DNA (RAPD) of these techniques can be used to detect genotoxicity and differences in RAPD profiles and can clearly be shown when comparing DNA fingerprints from untreated and treated individuals to genotoxic agents [Atienzar et al. 2002; Enan 2006; Cenkci et al. 2009].
The aim of the present study was to evaluate the DNA changes induced by toxic chemicals containing different metals (Cu, Mn, Pb, Ni, Cd and Zn) using RAPD technique in the plant model system treated with two selected concentrations and to investigate if plant bioassays can effectively detect the genotoxic effects of toxic metals and might be useful for biomonitoring.
The common bean, *Phaseolus vulgaris L.* (*Fabaceae*) was used as plant material. This plant is exposed to heavy metals in his natural environment as a result of various human activities. Plant seedlings were growth in sterile vitro containers containing liquid medium (untreated control treatment) [Murashige and Skoog 1962] or supplemented with $CuSO_4{:}5H_2O$, $MnSO_4{:}H_2O$, $Pb(NO_3)_2$, $NiSO_4$, $Cd(NO_3)_2$ and $ZnSO_4{:}7H_2O$ as treatment solutions. The seedlings were treated with the sollution of toxic chemicals at the concentrations of 150 mg L^{-1} and 350 mg L^{-1} for 7 days in a growth chamber with a 16 h photoperiod, with light intensity of $\mu E\ m^{-2}s^{-1}$. DNA extractions were performed using REDExtract-N-Amp Seed PCR Kit (Sigma-Aldrich). Amplifications were performed in a DNA thermocycler (Mastercycler personal, Eppendorf) using 5 steps PCR-protocol [Enan MR, 2006].
The marked changes observed in RAPD profiles (disappearance and/or appearance of bands in comparison with untreated control samples) were evaluated. Numerical analysis based on banding pattern obtained from treated samples was compared with the untreated sample (control) via hierarchical cluster analysis using NTSYSps (Numerical Taxonomy and Multivariate Analysis System) program with SAHN module [Rohlf 1994]. RAPD profiles generated by treated samples were different from those obtained using control DNA. The samples treated with 350 mgL^{-1} of toxic metals yielded a large number of new fragments (total 11) compared with total number of new fragments (total 5) at 150 mgL^{-1}. Similarly, the total number of disappeared fragments was 7 at 350 mgL^{-1}, whereas at 150 mgL^{-1}, the number of disappeared bands was 5. The highest number of missing bands was observed in samples treated with zinc (total 4 bands) and nickel (total 4 bands) at both concentrations. The highest number of appearance of new fragments (total 3) is recorded in DNA samples treated with copper at concentration 350 mgL^{-1}. In our study, the number of lost bands was found higher than that of extra bands. The disappearance of normal bands may be related to the DNA damage (e.g. single-strand breaks, double-strand breaks, modified or oxidized bases, bulky adduct), point mutations and/or complex chromosomal rearrangements induced by genotoxic chemicals [Atienzar and Jha 2006].
The presence in the DNA "fingerprint" of any variable RAPD profiles can be evidence for genotoxicity and used for hazard identification of environmental pollutants like xenobiotics. Plant bioassays can effectively detect the genotoxic effects and might be useful tool for biomonitoring.

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