RAPD-PCR Based Evaluation of Genotoxic Influence of Metal Stressors in Plant Model Systems

Darinka Gjorgieva Ackova, Katarina Smilkov, Viktorija Maksimova

Department of Applied Pharmacy, Division of Pharmacy, Faculty of Medical Sciences, Goce Delčev University, Štip, R. North Macedonia

Introduction

Among contaminants in tissues and environmental samples, metals are always in focus because of possible detrimental and long-lasting effects on living organisms. Plants are unique systems in their ability to serve as *in situ* monitors for environmental genotoxins.

The presented study was designed to get the possibility to measure the outcome of long-term exposition of high concentrations of different metal stressors on DNA damage in plant model systems.

Experimental

We used different plant species as models, like *Taraxacum officinale* L. (Asteraceae), *Matricaria recutita* L. (Asteraceae), *Robinia pseudoacacia* L. (Fabaceae), and *Urtica dioica* L. (Urticaceae). Plant samples were collected from two different areas, metal polluted (area around city of Veles) and referent area (without metal exposition, Plačkovica Mountain). Metal contents (cadmium, lead, copper, nickel, and zinc) in the samples was determined by using ICP-AES technique.

DNA extraction (frozen plant samples were used for DNA isolation by using REDExtract-N-Amp Plant PCR Kit (Sigma-Aldrich)) and RAPD-PCR analysis by using seven different primers (with 60-70 % GC content), were performed with all samples. RAPD profiles of plants exposed to metal stress and control plants (non-exposed) were compared.

Fig.1. Schematic representation of RAPD-PCR analysis

Results

Agarose-gel electrophoresis reveal total of 37 bands with molecular weights ranging from 1250 to 5000 bp (Table 1). Distinctive polymorphism of 72.97% (27 bands) total in all plant species investigated was estimated. The dendrogram constructed (Fig. 2) using NTSYSpc programme, showed that there is grouping in separate clusters of the same plant model collected from two different areas (metal-exposed and control samples).

Table 1. Changes in the RAPD profiles of the investigated plants, where "+" is for new appearance of DNA bands, and "-" is for disappearance of DNA bands in comparison with control profile.

Plant model system	Changes in the RAPD profile (in total with primers set used)	
	+	-
Urtica dioica	12	1
Matricaria recutita L.	1	3
Robinia pseudoacacia L.	5	2
Taraxacum officinale	3	0
Total	**21**	**6**

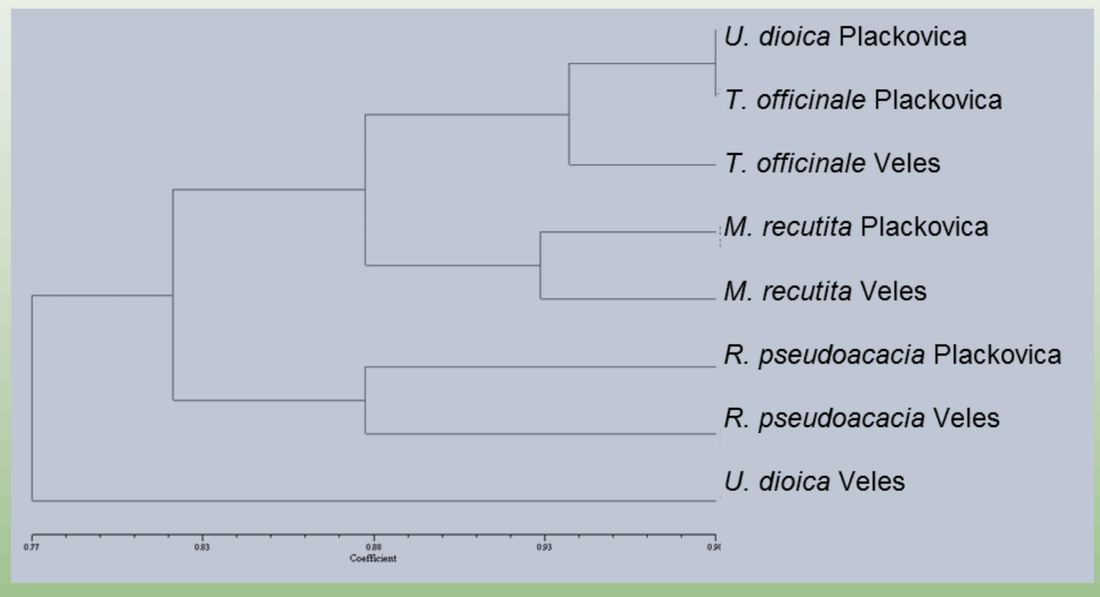

Fig. 2. Dendrogram based on detected DNA polymorphism in the investigated plants model systems.

Conclusions

The number of polymorphic bands observed in samples exposed to metals suggests that long term metal-exposition in high doses can cause mutations on genomic level in investigated model plants. These bands are unique and distinctly differentiated the samples and can act as markers for evaluation of the environmental metal exposition. Encounter the fact that plants are used as food or in medical purposes, the issue of possible genotoxicity initiated by metal contamination must be concerned.

Trace metal metabolism in plants, COST Action 19116, Ankara, Turkey, 2022