Chromium induced and its detection by RAPD in cotton (*Gossypium hirsutum.* L) plant

D.Ezhilvannan[1st] P.S.Sharavanan [2nd] K.Chitra [3rd]

[1]Ph.d Research Scholor.Bharathiar University-641046
*[2]Department of Botany, Annamalai University - 608002,*Deputation MRG Govt. Arts College, Mannargudi,*
[3]Department of Botany,Botany,Bharathiar University. Coimbatore-641046 Tamilnadu, India.

***Abstract*:**

The study was made to know the polymorphism of the cotton plants treated with different concentrations of chromium metal using RAPD analysis. The result revealed that using of the 10 primers only one primer detected 62 bands but remaining primers were found none of the bands. Among the 62 bands detected primer, 56 bands indicated monomorphic bands and rest of which bands showed the polypolymorphic for tested plants. In comparison, 1500bp obviously detected the prominent bands in all the wells than other ones.

Keywords: *Gossypium hirsutum*, RAPD, PCR, Chromium.

Introduction

Phytoremediation is an alternative or complimentary technology that can be used along with or in some cases in place of mechanical conventional clean-up technologies that often require high capital inputs and are labor and energy intensive; phytoremediation is an *in situ* remediation technology that utilizes the inherent abilities of living plants[1]. It is also an ecologically friendly, solar-energy driven clean up technology, based on the concept of using nature to cleanse nature. Contamination of soil by oil spills is a wide environmental problem that often requires cleaning up of the contaminated sites[2]. Phytoremediation is a broad term that has been used since 1991 to describe the use of plants to reduce the volume, mobility or toxicity of contaminants in soil groundwater or other contaminated media[3]. Phytoremediation uses plants to clean up pollution in the environment. Plants can help clean up many kinds of pollution including metals, pesticides, explosives and oil. The plants also help prevent wind, rain and groundwater from carrying pollutants away from sites to other areas. Phytoremediation is a non-destructive and cost effective *in situ* technology that can be used for the cleanup of contaminated soils[4]. The potential for this technology in the tropics is high due to the prevailing climatic conditions which favors plant growth and stimulates microbial activity[5]. The ability of plants to remove contaminants from the environment has been recognized and taken advantage of in application such as land farming of waste. The plant has evolved to the construction of treatment wetlands or even the planting of trees to counteract air pollution[6]. In more recent years as recognition grew of the damage resulting around the world from decades of an industrial economy and extensive use of chemicals and interest in funding technologies that could address the residual contamination among them phytoremediation. In the present work was aimed to the metal accumulation capacity of cotton plant was studied in *in situ* and *ex situ* condition under chromium stress and the effect of this metal of such a plant was studied by RAPD techniques.

Materials and Methods

Gossypium hirsutum L. seeds were obtained from Tamil Nadu Agricultural University (TNAU), Coimbatore. The seeds are uniform size and colors were selected for the experimental purpose. The different treatments of chromium *viz.*, control (normal soil), 5, 10, 15, 20 and 25 mg/kg soil selected seeds were sown in the pots to irrigate with normal tap water.

DNA sample preparation:

Genomic DNA was isolated from the young leaves of cotton plants by the standard CTAB method. Quantification of DNA was performed both spectrophotometrically and electrophoretically using Lambda DNA digested with Hind III as a standard marker.

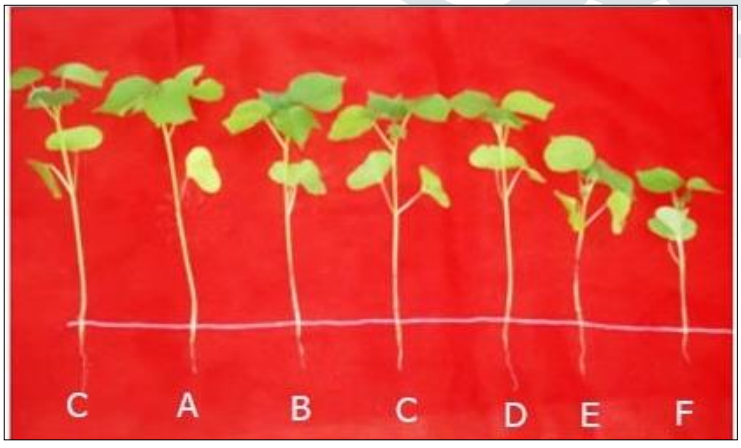
PCR condition:

PCR was carried out in a total volume of 25µl containing 2.5µl of 10x assay buffer (100mM Tris-HCL, pH 8.3, 500mM KCL), 2.5mM $MgCl_2$,200µM of each nucleotides, 25µmol of respective primer (Operon Technologies), 50ng of template DNA and 1unit of Taq polymerase (Biogen Biotech Pvt. Ltd). The PCR condition consisted of initial denaturation step for 5 min at 94^0C followed by 35cycles of 1 min at 94^0C, 1 min at 37^0C and 1 min 30 sec at 72^0C; and a final extension 10 min at 72^0C. PCR products were analyzed by electrophoresis in a 1.5% agarose gel in 1 x TBE and then visualized using Ethidium bromide staining.

Results and discussion

Characterization of the genetic variation is the one of the evidence for same are present or not in metal treated cotton plant (Fig 1). 10 primers were selected because wide ranges of available RAPD primers were used to study the genetic variability in the *Gossypium* unexpectedly 9 primers out of 10 was found failed. Remaining one primer was produced totally 62 bands. The prominent bands were detected in 1500bp in all wells. Of 56 bands were indicated monomorphic and remaining 6 bands were present polymorphic for these tested samples (Fig 2). The similar studies was found in alligator weed plant and the RAPD profiles of samples collected from the experiment sites showed no polymorphisms (except OPA 10 primer) was indicating that the plants having no genetic variation[7]. Therefore, its conducted with the intention of chromium creates genotoxic effect and induce DNA damage in plants and it can be observed by RAPD technique. In spite of having DNA alteration of this cotton plant can survive chromium induced contaminated soil and accumulates a certain amount of chromium proving its metal tolerant capacity

Fig 1 Morphological characteristics of chromium treated *G. hirsutum*



C. Marker(1Kb Ladder)

A. 10 ppm

B. 25pp

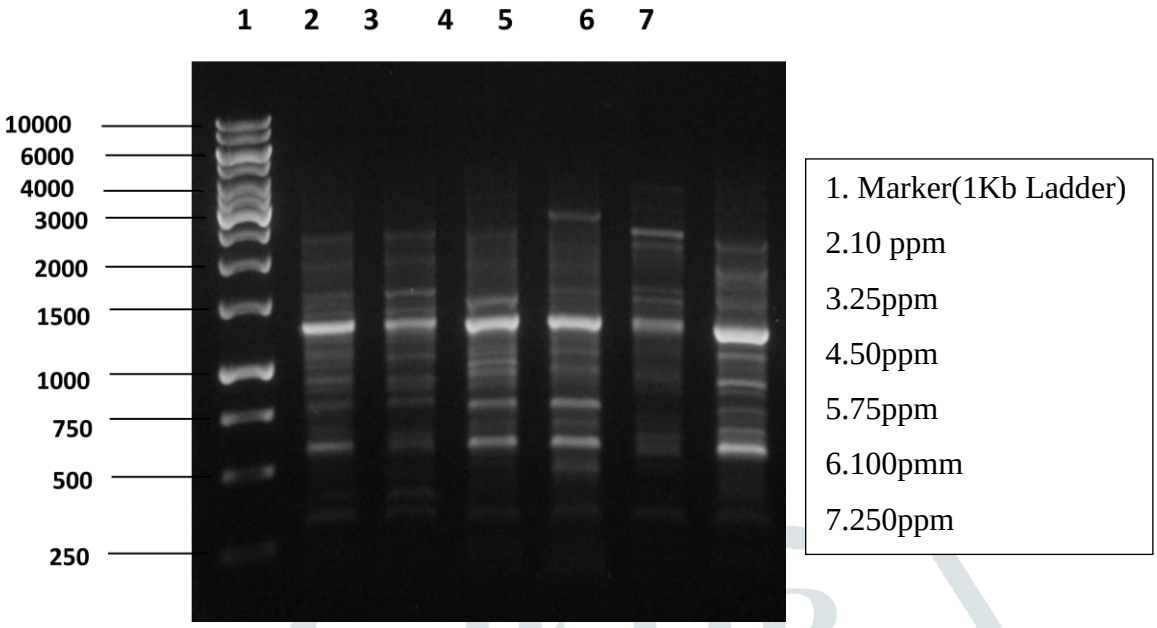
C. 50ppm

D. 75ppm

E. 100pmm

F. 250ppm

Fig 2 RAPD Analysis of *G. hirsutum*



Reference

1. Aslam, R., Ansari, M. Y. K., Choudhary, S., Bhat, T. M. and Jahan, N., 2014, Genotoxic effects of heavy metal cadmium on growth, biochemical, cyto-physiological parameters and detection of DNA polymorphism by RAPD in *Capsicum annum* L- an important spice crop in India. *Saudi J bio Sci*, 21: 465-472.

2. Bundy, J. G., Paton, G. I., and Campbell, C. D. 2002. Microbial communities in different soil types do not converge after diesel contamination. *J. Appl. Microbiol*., 92: 276-288.

3. Gopal, R., and Khurana. N., 2011, Effect of heavy metal pollutants on sunflower, *Afr J plant sci* 5(9); 531-536. Metal stress in plants-From molecules to Ecosystem. Spinger, Berlin; 1-27

4. Sethi, P. K., and Khandeiwal, D. J., 2006.Cadmium exposure: health hazards in developing countries, *Med Toxicol*, 2, 14-15.

5. Suparna and Rita kundu, 2015.The similar studies was found in alligator weed plant and the RAPD profiles of samples collected from the experiment sites showed no polymorphisms except OPA 10 primer) was indicating that the plants having no genetic variation, *International Journal of Engineering Technology Science and Research*, 2 (9), 1-11.

6. USPEA, 2000, Public Comment Draft. "Proposed Guidance on Cumulative Risk Assessment of Pesticide Chemicals that Have a Common Mechanism of Toxicity, Office of prevention, pesticides and toxic substances, Washington, DC.

7. Zhang H., Kim M. S., Sun Y., Dowd S. E., Shi H., Paré P. W. 2008. Soil bacteria confer plant salt tolerance by tissue-specific regulation of the sodium transporter HKT1. *Mol. Plant Microbe Interact.*21 737–744.