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Evaluation of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage in mosquito larvae treated with plant extracts

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ABSTRACT

The random amplified polymorphic DNA (RAPD) assay was used to assess the level of DNA damage in various exposed and unexposed *Culex quinquefasciatus* larvae to acetone and chloroform extracts of *Curcuma longa* and *Melia azedarach* at different concentrations (6.25, 12.5 and 25 ppm). This is the first report of an analysis of genomic alterations in plant extracts-treated mosquito larvae using RAPD-PCR fingerprinting. In comparison to the control larvae, larvae treated with the plant extracts caused greater changes in the RAPD patterns. DNA strand breakage was more in the larvae of *C. quinquefasciatus*.

Key words: RAPD; PCR; DNA damage; plant extracts; mosquito.

INTRODUCTION

Organisms when continuously exposed to environmental stress may result in DNA damage. The explorations of random amplified polymorphic DNA (RAPD) as genetic markers have improved the detection of DNA alterations after the influence of many genotoxins.[1] RAPD-PCR is one of the most reliably used techniques for detecting DNA damage as the amplification stops at the site of the damage. The changes occurring in RAPD profiles following genotoxic treatments include variation in band intensity as well as gain or loss of bands. This has been done through the analysis of band intensities and/or band gain/loss variation between exposed and non-exposed individuals. Indeed, the gain/loss or intensity differences of RAPD bands may be related to DNA damage, mutations or structural rearrangements induced by genotoxins, affecting the primer sites and/or interpriming distances.[2]

The RAPD method has recently been applied to detect genetic instability in tumors[3] and successfully detected genomic DNA alterations induced by several DNA damaging agents, such as benzopyrene[4], heavy metals[5] and UV radiation.[6] The final purpose of the present work was to identify the possible mo-

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lecular site or gene-specific markers linked to biopesticides. In the present study, third instar larvae of *C. quinquefasciatus* Say (Culicidae) exposed to acetone and chloroform extracts of *Curcuma longa* L. (Zingiberaceae) and *Melia azedarach* L. (Meliaceae) were used to screen genome-wide DNA alterations by using RAPD-PCR method.

MATERIALS AND METHODS

Mosquito culture

Eggs and larvae of *C. quiquefasciatus* were reared in plastic and enamel trays with tap water. They were maintained at 27±2°C and 75-85% relative humidity under 14:10 light and dark cycles.[7]

Preparation of plant extracts

Acetone and chloroform extracts of *C. longa* and *M. azedarach* at different concentrations (6.25, 12.5 and 25 ppm) were prepared by cold percolation.[8] Leaves were air-dried under shade and ground into fine powder using electric blender. 50 gm of dried powder were soaked in 300 ml of acetone and chloroform for 48 h with intermittent shaking. The plant extracts were filtered through Whatman No. 1 filter paper into conical flask. The filtrates were dried until a constant dry weight of each extract was obtained. The residues were stored at 4°C for further use. The volume of stock solution was 20 ml of 1%, obtained by weighing 200 mg of the plant extract and adding 20 ml ethanol to it.

DNA extraction

DNA from mosquito larvae was extracted by the method of Ballinger-Crabtree *et al*.[9] with slight modification. Ethanol preserved specimens were ground in 200 µl lysis buffer (100 mM Tris-HCl, pH 8.0; 1% sodium dodecyl sulphate; 50 mM NaCl; 50 mM EDTA), and the mixture was treated with 5 µl of proteinase K (20 mg/ml) for 16 h at 37°C. The suspension was extracted twice with equal volume of phenol-chloroform, and DNA was extracted by the adding 0.2 volumes of 5 M NaCl and 2.0 volumes of ethanol at room temperature. The mixture was incubated overnight at -20°C and spun at 12,000 rpm for 10 min to get pellet which was resuspended in 100 µl of sterilized distilled water and stored at 4°C. DNA concentrations were determined by spectrophotometeric analysis.

RAPD-PCR

RAPD amplification was done with a 15 µl PCR mix, containing 1x PCR buffer 1.5 µl, $MgCl_2$ (1 mM) 0.6 µl, dNTP (0.2 mM) 0.3 µl, BSA (0.533µl/ml) 0.8 µl, primers (MA-09, MA-12 and MA-26; Table 1) 0.3 µl, 1.5 unit of Taq (0.3 µl) and filled up with sterile deionized water to the final volume. 1 µl of extracted DNA was also added in each PCR tube. Three primers were randomly selected for RAPD analysis. The reaction mixture was given a short spin for thorough mixing of the cocktail components. PCR tubes were loaded on to a thermal cycler. The PCR involved an initial denaturation step at 94°C for 4 mins followed by 45 cycles with 94°C for 1 min for DNA denaturation, annealing as mentioned with each primer, extension at 72°C for 2 minutes and final extension at 72°C for 10 minutes were carried out.

Table 1. Random primers used for RAPD analysis.

Primer	Sequence (5′ to 3′)	Annealing Tm °C/sec
Primer MA-09	GACGGATCAG	32
Primer MA-12	ACCGCGAAGG	34
Primer MA-26	GACGTGGTGA	32

Agarose gel electrophoresis

The amplification products were analyzed

by electrophoresis.[10] Along with the PCR amplified products, 100 bp DNA ladders as standard marker were subjected to electrophoresis in 1.5% agarose gel in TAE buffer and stained with ethidium bromide. Molecular size of the marker was 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp. The amplified pattern was visualized on a UV transilluminator and photographed. Polymorphism was evidenced as the presence and/or absence of DNA fragments between the samples. The RAPD profiles of the treated insects were evaluated on the same 1.5% agarose gel run for 30 min at 120 volts.

RESULTS AND DISCUSSION

The extracted DNA samples of 3rd instar *C. quinquefasciatus* larvae exposed to acetone and chloroform extracts of *C. longa* and *M. azedarach* at different concentration (6.25, 12.5 and 25 ppm) were further evaluated for their DNA changes in comparison with untreated control larvae. Different range of DNA modifications was observed in the treated larvae in comparison with untreated control. Number of bands was increased in the *M. azedarach* (acetone extracts) treatment whereas in *C. longa* acetone and chloroform treatments the number of bands were decreased (Table 2, Fig. 1). Further, it was observed that DNA strand breakage was more in the larvae of *C. quinquefasciatus* treated with plant extracts.

This is the first report of an analysis of genomic alterations in plant extracts-treated mosquito larvae with the use of RAPD-PCR fingerprinting. Modifications of the RAPD patterns can be due to changes in primer binding sites, structural changes due to DNA damage. The interpretation of the molecular events responsible for differences in the RAPD patterns is not an easy task since different DNA alterations can induce similar type of changes. RAPD analysis allows a qualitative assessment of the DNA effects and the nature of the changes in profiles can only be speculated unless amplicons are analyzed with sequencing, probing etc.

RAPD assays have been shown to detect DNA damage caused by selected plant ex-

Table 2. Primers used for RAPD amplification of plant extract treated genomic DNA from larvae of *C. quinquefasciatus.*

Primer	Plant extract	Solvent used	Concentration (ppm)	Total bands
MA-09	Control			7
	Curcuma longa	acetone	25	8
		chloroform	6.25	6
			12.5	2
	Melia azedarach	acetone	12.5	10
MA-12	Control			6
	Curcuma longa	acetone	25	5
		chloroform	6.25	8
			12.5	6
	Melia azedarach	acetone	12.5	10
MA-26	Control			6
	Curcuma longa	acetone	25	2
	Curcuma longa	chloroform	6.25	2
			12.5	smear
	Melia azedarach	acetone	12.5	11

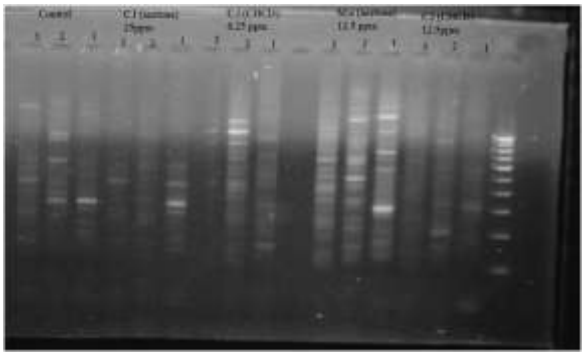

Figure 1. Comparison of RAPD-PCR profiles of 3rd instar larvae of *C. quinquefasciatus* control and treatment regimes with acetone and chloroform extracts of *C. longa* and *M. azedarach*. Lane on the right side is the DNA size marker (100 bp). 1 - Primer MA-09; 2 - Primer MA-12; 3 - Primer MA-26; C.l - *C. longa*; M.a = *M. azedarach.*

tracts at different doses. The changes occurring in RAPD profiles treatments include variation in band intensity as well as gain or loss of bands. Exposure of an organism to a these plant extracts may result in the formation of covalently bound adducts between the chemical or its metabolites and the DNA; faulty repair of these adducts often results in mutations and, sometimes, cytogenetic changes. In addition, Jones and Kortenkamp[11] demonstrated that genomic alterations can only be picked up by the RAPD assay if they affect at least 2% of the cells. It was found that RAPD polymorphism due to mosquito larvae DNA exposed to mosquitocidal plant extracts can be distinguished from the untreated mosquito larvae.

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REFERENCES

1. Atienzar FA, Cheung VV, Jha AN & Depledge MH (2001). Fitness parameters and DNA effects are sensitive indicators of copper-induced toxicity in *Daphnia magna*. *Toxicol Sci*, 59, 241-250.
2. Atienzar FA, Venier P, Jha AN & Depledge MH (2002). Evaluation of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage and mutations. *Mutat Res,* 521, 151-163.
3. Papadopoulos S, Benter T, Anastassiou G, Pape M, Gerhard S, Bornfeld N, Ludwig WD & Dorken B (2002). Assessment of genomic instability in breast cancer and uveal melanoma by random amplified polymorphic DNA analysis. *Int J Cancer*, 99, 193-200.
4. Atienzar FA & Jha AN (2004). The random amplified polymorphic DNA (RAPD) assay to determine DNA alterations, repair and transgenerational effects in B[*a*]P exposed *Daphnia magna*. *Mutat Res*, 552, 125-140.
5. Enan MR (2006). Application of random amplified polymorphic DNA (RAPD) to detect the genotoxic effect of heavy metals. *Biotechnol Appl Biochem*, 43, 147-154.
6. Kumar A, Tyagi MB & Jha PN (2004). Evidences showing ultraviolet-B radiation-induced damage of DNA in cyanobacteria and its detection by PCR assay. *Biochem Biophys Res Commun,* 318, 1025-1030.
7. World Health Organization (2005). Guidelines for laboratory and field testing of mosquito larvicides. World Health Organization communicable disease control, prevention and eradication, WHO pesticide evaluation scheme. (WHO/CDS/WHOPES/GCDPP/2005.13).
8. DuraipandiyanV, Ayyanar M & Ignacimuthu S (2006). Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC Complement Altern Med*, 6, 35.
9. Ballinger-Crabtree ME, Black WC & Miller BR (1992). Use of genetic polymorphisms detected by the random-amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) for differentiation and identification of *Aedes aegypti* subspecies and populations. *Am J Trop Med Hyg*, 47, 893–901.
10. Sambrook J, Fritsch EF & Maniatis T (1989). *Molecular Cloning. A Laboratory Manual, 2 edn*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
11. Jones C & Kortenkamp A (2000). RAPD library fingerprinting of bacterial and human DNA: applications in mutation detection, *Teratogen. Carcinogen Mutagen,* 20, 49-63.