Arch. Biol. Sci., Belgrade, 67 (1), 235-239, 2015 DOI:10.2298/ABS140903029E

USE OF RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) TO DETECT DNA DAMAGE INDUCED BY *PRANGOS FERULACEA* (UMBELLIFERAE) ESSENTIAL OIL AGAINST THE MEDITERRANEAN FLOUR MOTH *EPHESTIA KUEHNIELLA* ZELLER (LEPIDOPTERA: PYRALIDAE)

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Abstract – The random amplified polymorphic DNA (RAPD) assay is a useful method for detecting genotoxin-induced DNA damage. In the present study, this assay was evaluated to measure the essential oil of *Prangos ferulacea*-induced DNA changes in *Ephestia kuehniella* Zeller larvae. The third instar larvae of *E. kuehniella* were exposed to 500 μl L^{-1} essential oil for 24 h. Forty random primers were used for RAPD-PCR of which eleven produced unique polymorphic band patterns. In comparison with control larvae, the essential oil-treated larvae showed greater changes in RAPD profiles. This is the first report of an analysis of the genotoxic effect of *P. ferulacea* essential oil against *E. kuehniella* larvae using RAPD-PCR.

Key words: RAPD-PCR; *Ephestia kuehniella*; *Prangos ferulacea*; essential oil; DNA damage

Received September 3, 2014; **Revised** September 17, 2014; **Accepted** November 6, 2014

INTRODUCTION

The random amplified polymorphic DNA (RAPD) assay based on PCR, developed by Williams et al., (1990), amplifies random DNA fragments with short primers of arbitrary nucleotide sequence. This technique has been reported as an effective method for species classification, genetic mapping and phylogeny studies (Williams et al., 1990). It has been used to detect DNA damage and mutations in different organisms (Savva, 1998; Atienzar et al., 2001).

DNA damage induced by some chemicals, such as benzo[a]pyrene (Atienzar et al., 1999), mitomycin (Becerril et al., 1999), copper (Atienzar et al., 2001) and physical agents, like UV and X-ray (Kuroda et al., 1999) were successfully detected by RAPD-PCR. After genotoxic treatments, changes in RAPD profiles included variation of band intensity as well as gain or loss of bands. These differences of band profiles may be related to DNA damage, mutations or structural rearrangements induced by genotoxins (Atienzar et al., 2002a).

The Mediterranean flour moth, *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) is a common, cosmopolitan pest of cereal mills. It has been widely used as a host for rearing of parasitoids for biological control (Tavares et al., 1989). Adult moths do not need to feed before laying eggs but larvae of moths produce webbing particles, feed and make food inconsumable (Cox et al., 1991).

In the present study, third instar larvae of *E. kuehniella* exposed to essential oil of *Prangos ferulacea* (Umbelliferae) and the genotoxic effect of the essential oil were screened using the RAPD-PCR method. Generally, control of this pest is done by regular treatment of infested areas with pesticides, such as malathion, dichlorvos and methyl bromide (Tuncbilek et al., 2009). At the same time, the insecticidal effect of environmentally safe methods, such as using the essential oils of plants, magnetic fields and carbon dioxide treatments on *E. kuehniella* have been reported (Ayvaz et al., 2009, Karabörklü et al., 2011, Ercan et al., 2013, Pandır et al., 2013a, Pandır et al., 2013b).

Magnetic field-induced DNA damage in *E. kuehniella* larvae were shown by Pandir and Sahingoz, (2014), using the comet assay. Tunçbilek et al. (2011) demonstrated gamma-induced DNA damage in *E. kuehniella* with the same technique. In a nutshell, there have been no reports on the genotoxic effect of *P. ferulacea* essential oil on *E. kuehniella* larvae using RAPD.

The aim of this study was to detect the essential oil of *P. ferulacea*-induced DNA damage in *E. kuehniella* larvae by using RAPD-PCR, comparing changes in RAPD profiles of non-treated (control) and treated (essential oil exposed) samples.

MATERIALS AND METHODS

Ephestia kuehniella culture

Ephestia kuehniella Zeller adults were obtained from the Adana Plant Protection Research Institute. They were reared on a mixture of 1 kg wheat flour, 55 g yeast, and 30 g of wheat germ (Marec et al., 1999). Larvae were from laboratory stock cultures and reared at 27±1°C and 70±5% relative humidity and under a light regime of 14 h light followed by 10 h dark cycles (Ercan et al., 2013).

Plant Material

Aerial parts of *Prangos ferulacea* (Umbelliferae) were collected at the flowering and fruit stages from the area between Tunceli and Pulumur in Turkey at an altitude of 1 210 m in July 2011. They were enumerated as M. Koç 1334. Samples were pressed and dried according to herbarium techniques, identified by *The Flora of Turkey* (Davis, 1972), and kept at the herbarium of the Bozok University Department of Biology (Yozgat, Turkey). Isolation and analysis of the essential oil were applied according to the method of Ercan et al. (2013).

Essential oil application

Third instar larvae of *E. kuehniella* were exposed to 500 µl L^{-1} air essential oil for 24 h. Selection of this concentration and application were based on our earlier study (Ercan et al., 2013). Control and treated larvae were used for DNA isolation and used for generation of RAPD profiles.

DNA isolation and RAPD-PCR

DNA isolation was performed by using EZNA insect DNA isolation kit. The concentration of DNA was estimated using UVS-99 UVISDrop Spectrophotometer (ACTGene). RAPD-PCR was performed in reaction mixtures of 15 µl, containing PCR buffer 1.5 µl (10X buffer with $(NH_4)_2SO_4$, Fermentas), $MgCl_2$ (2.5 mM, Fermentas) 1.2 µl, dNTP (10 mM stock solution) 0.5 µl, BSA (10 mg/ml) 0.6 µl, primers (10µM, Bio Basic Inc.) 1.0 µl, Taq Polymerase (5 u/µl, Fermentas) 0.25 µl and filled up with sterile deionized water to the final volume. 2 µl of extracted DNA was also added to each PCR tube. For amplification, reaction mixtures were denaturated at 94°C for 2.5 min. followed by 36 cycles consisting of denaturation for 45 s at 94°C, primer annealing for 45 s at 35°C and extension for 2 min at 72°C and final extension at 72°C for 10 min.

Agarose gel electrophoresis

The amplification products were electrophoresed in a Tris-Acetic Acid-EDTA buffer by 1% agarose gel at 80V for 1.5 h stained with ethidium bromide, and RAPD profiles were visualized under UV on a UV

Table 1. Random primers that gave polymorphic bands in RAPD-PCR.

Primer	Sequence of primer (5′→3′)
S1	GTTTCGCTCC
S5	TGCGCCCTTC
S15	GGAGGGTGTT
S17	AGGGAACGAG
S24	AATCGGGCTG
S25	AGGGGTCTTG
S52	CACCGTATCC
S53	GGGGTGACGA
S55	CATCCGTGCT
S57	TTTCCCACGG
S97	ACGACCGACA

Table 2. RAPD profile variance of the number of bands determined with 11 primers in essential oil-treated *E. kuehniella* larvae compared with non-treated larvae.

Primers	Control	Essential oil application (500 µl L^{-1} air)			
		a	b	c	d
S1	8	0	5	2	1
S5	4	1	1	3	0
S15	6	0	0	1	2
S17	10	3	0	1	1
S24	5	0	0	4	0
S25	5	2	1	0	1
S52	4	0	1	1	0
S53	3	1	0	0	0
S55	7	2	2	2	2
S57	3	2	3	0	0
S97	3	2	1	2	0
a+b		27			

*a) appearance of extra bands; b) disappearance of normal bands; c) decreasing of band intensity; d) increasing of band intensity (a+b: polymorphic bands)

transilluminator and photographed. Along with the PCR amplified products, a 100 bp DNA ladder was used as standard marker. Forty oligomers (Bio Basic Inc.) that contain 10 nucleotides were used for amplification reactions. The sequences of oligomers that gave polymorphic bands are shown in Table 1.

RESULTS AND DISCUSSION

The third instar larvae of *E. kuehniella* were exposed to a lethal dose of *P. ferulacea* essential oil. The concentration of essential oil was adjusted according to our previous study (Ercan et al., 2013). Control and treated larvae were used for DNA extraction for RAPD analysis. After amplification, DNA changes were compared. Polymorphic bands were divided into four types: 1) appearance of extra bands, 2) disappearance of normal bands, 3) decreasing of band intensity, and 4) increasing of band intensity (Table 2).

RAPD band profiles showed differences between treated and non-treated larvae with changes in number and size of amplified DNA fragments. It was observed that a number of RAPD bands were changed in treated larvae. Of the forty random decamer primers tested, only eleven of them gave specific and consistent bands. Extra bands appeared with primer S5 and primer S53 (one new band), primer S25, S55, S57 and S97 (two new bands) and primer S17 (three new bands). The highest number

of bands disappearing was observed in primer S1 (Table 2). In primer S24, extra band occurrence or disappearance of band were not observed but the maximum number of band intensity decrease occurred in this primer. In the study, the number of lost bands was found to be higher than that of new bands. At the same time, the occurrence of band intensity decreasing was higher than that of intensity increasing. The loss of normal bands may be related to the DNA damage, point mutations or complex chromosomal rearrangements induced by genotoxic factors (Atienzar and Jha, 2006).

Eleven primers gave a total of 58 bands between control and treated larvae. The polymorphism value was 46.5 % and it was due to loss and/or gain of bands in the control compared with treated larvae.

The Random Amplified Polymorphic DNA (RAPD) method has enabled the development of a sensitive assay for detecting genotoxicity and DNA damage (Atienzar et al., 2002a, Conte et al., 1998, Atienzar et al., 2002b). Detection of genotoxic effects using this technique involves the comparison of banding profiles obtained from control and exposed DNA. RAPD is a PCR-based molecular marker that is sensitive, effective, cheap and relatively simple as it gives result about DNA damage after exposure to any physical and chemical agents (Atienzar et al., 2001).

Extra bands and an increase in band intensity could be ascribed to changes in DNA conformation (Atienzar et al., 2002b). Primer binding sites may change, resulting in different electrophoretic banding patterns (Savva, 1996).

With an adequate optimization of PCR conditions and selection of sensible primers for the DNA template, reproducible banding profiles can be obtained and used to detect DNA damage induced by genotoxins (Savva, 1998). In ecotoxicological studies, RAPD-PCR can be used as a powerful tool for detecting DNA damage, e.g., nitrofurazone-induced DNA damage has been detected by RAPD assay (Zhou et al., 2011).

The application of plant extracts on an organism may cause the creation of covalently bound adducts between a chemical or its metabolites and the DNA, and incorrect repair of these adducts may cause mutations and cytogenetic changes (Lalrotluanga et al., 2011). If genomic alterations affect at least 2% of the cells, RAPD-PCR can be used for detection of these alterations (Jones and Kortenkamp, 2000).

DNA damage in *E. kuehniella* was demonstrated by comet assay in different studies (Pandır and Sahingoz, 2014; Tunçbilek et al., 2011). In the present study, essential oil-treated larvae were subjected to DNA damage analysis by RAPD assay. DNA band variations were observed when *E. kuehniella* larvae were exposed to *P. ferulacea* essential oil. Changes in the RAPD profile revealed the effect of the essential oil on DNA integrity. Variances were defined by losses and/or gains of bands and alterations in band intensity. In conclusion, this study aimed to introduce the usefulness of RAPD-PCR in essential oil-induced DNA damage studies. The results demonstrated the potential of the RAPD assay as a powerful tool for detecting genotoxicity induced by essential oil vapor. This is the first report of *P. ferulacea* essential oil-induced DNA damage in *E. kuehniella* larvae detected by using RAPD-PCR.

Acknowledgments - I would like to thank Mrs Sevcan Oztemiz (Biological Control Research Station in Adana) for supplying the Ephestia kuehniella. Special thanks to Murat Koç for collecting the plant material.

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