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Genetic variation of *Pectinophora gossypiella* (Saunders) treated with some insecticides using polymerase chain reaction (PCR) technique

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Abstract

Random Amplified Polymorphic DNA (RAPD) was used to examine the pooled genomic DNA of *Pectinophora gossypiella* 4th instar larvae treated with recommended doses of various pesticides in the field strain of the pink bollworm (*Pectinophora gossypiella*). Insecticide-free cotton fields were used to collect a field strain of the pest. Four insecticides utilized in this study agristar, proclaim, tracer, and Camkron. A total of five primers were employed to examine the mutagenic differences between the treated strains. Most fragments were created with the HB-12 primer (10 fragments). Primers HB-11 and HB-12 have the fewest pieces, respectively (6 fragments). A total of seven to nine overlapping fragments were produced by the other three primers. Fragments varied in size from 180 to 1435 bp in molecular weight. One of the pink bollworm *P. gossypiella* 4th instar larvae treated with proclaim insecticide had a lower similarity index (0.38) than the untreated one, indicating the greatest degree of change in DNA structure and sequences between untreated pink bollworm larvae and those treated with various insecticides. The RAPD patterns that resulted from this comparison showed that Primers HB-11, HB-14, and HB-10 had the greatest similarity index (1.00) between treated populations and the control of the pink bollworm.

Key words: *Pectinophora gossypiella* , RAPD, DNA, primers, similarity index, fragments.

Introduction

Among the most devastating cotton pests in Egypt is the pink bollworm, *Pectinophora gossypiella* Saunders. During the period 1903-1913, it was brought into Egypt from India in poorly ginned cotton that included infested seeds (Willcooks, 1916). In 1913, the insect had spread to such an extent that Egypt's cotton harvest was in jeopardy. It causes severe damage to cotton bolls, resulting in a significant decrease in both quantity and quality of the cotton production. RAPD uses in genetic study of novel species without previous information about the specific DNA sequences or genes. In contrast to biochemical markers, which are confined to a few genomic areas, the RAPD-PCR approach potentially detects polymorphism throughout the whole genome, whether in coding or non-coding regions, and may therefore be used to any mutagen, such as insecticides. The pace of evolution of various regions of the genome might vary greatly (Nei, 1987). It's likely that PCR-amplified areas develop at a faster pace than those that weren't. It is thus possible to increase the variety of RAPD fingerprints by amplifying micro or mini satellite DNA in the amplified locations (Baruffi et al., 1993 and Abdel- baset, 2009).

Field-collected *P. gossypiella* fourth instar larvae treated with four different pesticides were subjected to a genetic variation research using the Random Amplified Polymorphic DNA (RAPD-PCR) method.

Materials and methods:

Insect used:

The 4th instar larvae of pink bollworm**,** *Pectinophora gossypiella* field population were collected from cotton field of Qaha farm of Syngenta company of insecticides which did not receive any insecticidal application were used in this investigation..

Insecticides used: all insecticides were applied at the recommended concentration

1.Common name: Spinosad.
Trade name **:**Tracer 24% SC, used at rate of 50ml/fed.

2. Common name: Emamectin benzoate.
Trade name :Proclaim 5% SG, used at rate of 60gm./fed.

3. Common name : Lambda-cyhalothrin.
Trade name : Agristar 5% EC, used at rate of 375ml/fed.

4.Common name :Profenofos.
Trade name : Camkron 72% EC , used at rate of 1L/fed.

Methods of application:

1.DNA isolation procedure

DNA from newly cut leaves was extracted using the DNeasy plant micro kit (bio basic). A UV spectrophotometer was used to measure the absorbance ratios A260/A280, which show that pure DNA has an A260/A280 ratio between 1.8 and 2.0. DNA samples were also tested for quality employing electrophoresis in a 1 percent agarose gel with bromheximide as an ethidume bromide.

2.Reaction of the Polymerase Chain

Polymerase Chain Reaction (PCR) amplification was carried out utilising five ISSR primers on genomic DNA. from Operon Technology, Alameda, U.S.A.: ISSR primers

According to Fathi *et al.* (2013), the amplification reactions for ISSR methods were carried out as specified in the following manner: To begin with, the reaction was held at 94 ^{o}C for 4 minutes, followed by 40 cycles of 1 minute at the mentioned temperature, 1 minute at the annulating temperature of 57 ^{o}C, for 2 minutes at 72^{o}C, and lastly 72^{o}C for 10 minutes.

Electrophoresis of 4 Gels

A 1.5 percent agarose gel containing ethidium bromide and 100 bp to 1kb ladder markers was used to differentiate amplified products. The run lasted around 30 minutes at 100 volts in a BioRad micro submarine gel.

Analysis of the 5-Gel sample

For each primer, gel analyzer3 software scored clear amplicons as either (1) present or (2) missing, and recorded the results as a binary data matrix. The DNA banding patterns were photographed using the Bio-1D Gel Documentation system. Adhikari *et al.* (2015). produced DNA-profiles using ISSR and SCOT methods on this matrix Analysis was done using XLSTAT.7 software utilising the agglomerative hierarchical clustering (AHC) approach developed from the unweighted pair-group average (UPGMA) method for the calculation of MD (Dissimilarity).

Table (1): List of the primer names and their nucleotide sequences used in the study

Number	Name	Sequence
1	A-14	CTC TCT CTC TCT CTC TTG
2	HB-10	GAG AGA GAG AGA CC
3	HB-11	GTG TGT GTG TGT TGT CC
4	HB-14	CTC CTC CTC GC
5	*HB-12*	*GAG GAG GAG C*

Results And Discussion

Random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) of field population of *Pectinophora gossypiella* 4th instar larvae treated with four insecticides:

Pooled genomic DNA from the *P. gossypiella* field population was screened using five primers with random sequences, as indicated in Table (1). HB-12, HB-10, and A-14 fingerprinting primers generated polymorphic and monomorphic fingerprints for the pests treated with four different insecticides, namely; agristar, proclaim, tracer, and Camkron, compared to untreated larvae; whereas the fingerprints generated by primer HB-1 were monomorphic for the treated and untreated larvae. These primers were effective in amplifying specific DNA segments. A total of 10 RAPD bands were detected using the primer HB-12, which was employed in the research for all treatments and pest management. Untreated larvae had 8, 10, 8, 7 and 8 bands, while treated larvae had 4, namely agristar, proclaim, tracer and Camkron, with four insecticides. The agristar-treated larvae shared 8 pieces of 1435, 830, 800, 670, 500, 420, 380, and 210 bp with the control larvae. The control larvae and treatment groups were exposed to amplified segments of 1435bp and 830bp, respectively. Larvae in the control and tracer-treated groups had access to the same five snippets of DNA. A total of six pb fragments ranging in size from 1435 to 210 pb were used in the experiments on both control and Camkron-treated larvae. Fragments 1435 and 540 pb were not present in the control sample, which ruled out their inclusion. Proclamation treatment resulted in the absence of bands 670 and 210 bp. With traces and Camkron, no 830 or 800 pb fragments were found in larvae. There were seven polymorphic and three monomorphic 4th instar larvae connected with the tested pest that were detected by primer HB-12 polymorphism.

Table (2): Total number and size of RAPD-PCR fragments generated by arbitrary primers in different treatments of *P. gossypiella* larvae using primer HB-12.

MW-bp	MW	Control	Agristar	Proclaim	Tracer	Camkron	Polymorphism
Band1	1435	1435	1435	1435	1435	1435	monomorphic
Band2	1325	-----	1325	1325	1325	1325	polymorphic
Band3	830	830	830	830	.-----	…….	polymorphic
Band4	800	800	800	800	…….	……	polymorphic
Band5	670	670	670	…….	670	670	polymorphic
Band6	540	-----	540	540	540	540	polymorphic
Band7	500	500	500	500	-----	500	polymorphic
Band8	420	420	420	420	420	420	monomorphic
Band9	380	380	380	380	380	380	monomorphic
Band10	210	210	210	-----	210	210	Polymorphic
Total	10	8	10	8	7	8	……

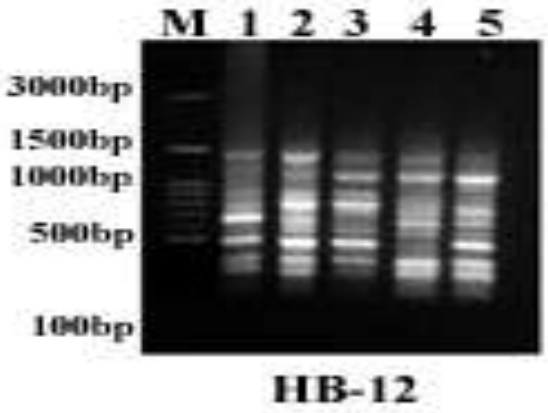

Fig.(1): RAPD-PCR produced for different treatments of *P. gossypilla* larvae using primer HB-12. Where is lane 1=Control, lane 2 =Agristar, lane 3=Proclaim, lane 4= Tracer and lane 5= Camkron.

Analysis of the similarity index showed a major drop from 0.93 to 0.67. In this respect highly resistance could be noticed in field population treated with tracer insecticide where the similarity index equal 0.67 (Table3).

Table (3): Estimated similarity index between different treated and untreated of *P. gossypiella* larvae using primer HB-12.

Treatments	Control	Agristar	Proclaim	Tracer	Camkron
Control	-----	0.89	0.75	0.67	0.75
Agristar	-----	-----	0.89	0.82	0.89
Proclaim	-----	-----	-----	0.67	0.75
Tracer	-----	-----	-----	-----	0.93
Camkron	-----	-----	-----	-----	-----

Using the primer HB-10, it was discovered that there was considerable difference in the efficacy of the various insecticides tested on the genetic variety of *P. gossypiella* 4th instar larvae. A total of seven RAPD-PCR bands were generated by amplification of 4th instar larval DNA using primer HB-10 as shown in (Table 4) and displayed in (Fig. 2). DNA fragments varied from four to six bands in number. Using primer HB-10, PCR patterns were generated that distinguished between the control and four insecticidal treatments for the pest's 4th instar larvae. All treatments and the control shared three amplified fragments, each measuring 945, 830, and 210 bp. To our knowledge, these two amplified pieces were only seen in the control and both treatments with proclaim and tracer insecticides. Only when the larvae were treated with proclaim and Camkron were fragments of 570 pb found in the laboratory strain. Three monomorphic and four polymorphic fingerprints were found in the fingerprints produced by the HB-10 primer. This primer resulted in 7 distinct bands of amplified DNA. Among the untreated and agristar-treated larvae, there were six, six, six, six, and four bands, respectively. Four of the amplified larvae had polymorphic polymorphism patterns, whereas the other three were monomorphic.

Table (4): Total number and size of RAPD-PCR fragments generated by arbitrary primers in different treatments of *P. gossypiella* larvae using primer HB-10.

MW-bp	MW	Control	Agristar	Proclaim	Tracer	Camkron	Polymorphism
Band1	1265	1265	1265	…….	1265	…….	Polymorphic
Band2	945	945	945	945	945	945	Monomorphic
Band3	830	830	830	830	.830	830	Monomorphic
Band4	680	680	680	680	680	680	Monomorphic
Band5	570	570	…….	…….	570	……	Polymorphic
Band6	465	465	465	…….	465	…….	Polymorphic
Band7	210	210	210	210	210	210	Monomorphic
Total	7	6	6	5	6	4	……

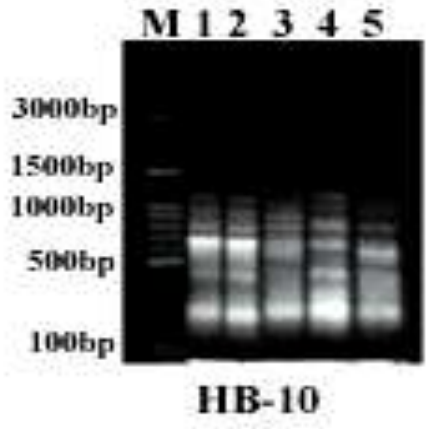

Fig.(2): RAPD-PCR produced for different treatments of *P. gossypilla* larvae using primer HB-10. Where ; lane 1=Control, lane 2 =Agristar, lane 3=Proclaim, lane 4= Tracer and lane 5= Camkron.

For the primer HB-10, similarity index values ranged from 0.60 to 1.00 in comparison with untreated larvae, demonstrating strong resistance in the fourth instar larvae treated with Camkron, as shown in (Table 5).

Table (5): Estimated similarity index between treated *P. gossypiella* larvae using primer HB-10.

Treatments	Control	Agristar	Proclaim	Tracer	Camkron
Control	-----	0.83	0.73	1.00	0.60
Agristar	-----	-----	0.73	1.00	0.60
Proclaim	-----	-----	-----	0.73	0.89
Tracer	-----	-----	-----	-----	0.60
Camkron	-----	-----	-----	-----	-----

Table (6) and Fig. (3) reveal that the primer BH-11 yielded the fewest bands, with a total of 6 fragments produced. There were 23 pieces found in the pink bollworm *P. gossypiella* larvae examined at the 4th instar stage. While there were only five pieces in the agristar and proclaim-treated and Camkron-treated larvae, six were found in the tracer insecticide-exposed larvae. Only in larvae treated with tracer was a fragment of 660 pb was found. Larvae in the control and those treated with the four pesticides studied included 600, 520, 440, 300, and 230 pb of fragmentation. Polymorphism patterns in the amplified larvae revealed one unique and five monomorphic patterns.

Table (6): Total number and size of RAPD-PCR fragments generated by arbitrary primers in different treatments of *P. gossypiella* larvae using primer HB-11.

MW-bp	**MW**	**Control**	**Agristar**	**Proclaim**	**Tracer**	**Camkron**	**Polymorphism**
Band1	660	……	…….	…….	660	…….	monomorphic
Band2	600	600	600	600	600	600	monomorphic
Band3	520	520	520	520	.520	520	monomorphic
Band4	440	440	440	440	440	440	monomorphic
Band5	300	300	300	300	300	300	monomorphic
Band6	230	230	230	230	230	230	monomorphic
Total	6	5	5	5	6	5	……

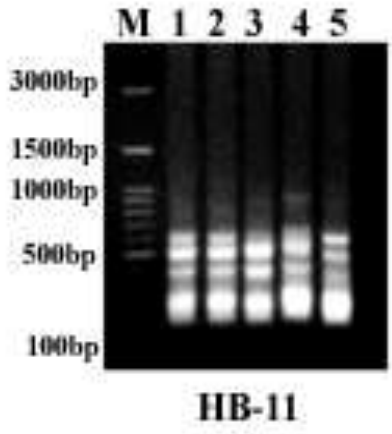

Fig.(3): RAPD-PCR produced for different treatments of *P. gossypilla* larvae using primer HB-11. Where ; lane 1=Control, lane 2 =Agristar, lane 3=Proclaim, lane 4= Tracer and lane 5=Camkron.

As summarized in Table (7), the similarity index of the treated larvae produced by the primer HB-11ranged from0.91 to1.00 compared with untreated one indicating low resistance levels in the tested larvae

Table (7): Estimated similarity index between treated *P. gossypiella* larvae using primer HB-11.

Treatments	Control	Agristar	Proclaim	Tracer	Camkron
Control	-----	1.00	1.00	0.91	1.00
Agristar	-----	-----	1.00	0.91	1.00
Proclaim	-----	-----	-----	0.91	1.00
Tracer	-----	-----	-----	-----	0.60
Camkron	-----	-----	-----	-----	-----

The RAPD-PCR patterns produced by amplification of *P. gossypiella* DNA with primer HB-14 contained a total of 9 bands (Table8 and Fig. 4). These fragments were 6,6,7,8 and 7 which appeared in the untreated larvae and larvae applied with agristar, proclaim, tracer and Camkron; respectively. Fragments of 1165, 685, 580, 400 and 360 pb were shared in the untreated 4th instar larvae and larvae treated with the previously mentioned tested insecticides. One fragment of 800pb was appeared only in larvae exposed to proclaim. One fragment of300 pb was detected only in larvae treated with tracer and Camkron, and one band of 230 pb was appeared only in larvae applied with proclaim and tracer insecticides. The fingerprints generated by primer HB-14 revealed one unique, 5 monomorphic and three polymorphic profiles.

Table (8): Total number and size of RAPD-PCR fragments generated by arbitrary primers in different *P. gossypiella* larval treatments using primerHB-14.

MW-bp	MW	Control	Agristar	Proclaim	Tracer	Camkron	Polymorphism
Band1	1165	1165	1165	1165	1165	1165	monomorphic
Band2	860	860	860	…….	860	860	Polymorphic
Band3	800	…….	…….	800	.-----	…….	Unique
Band4	685	685	685	685	685.	685	Monomorphic
Band5	580	580	580	580.	580	580	Monomorphic
Band6	400	400	400	400	400	400	Monomorphic
Band7	360	360	360	360	360	360	Monomorphic
Band8	300	……	……	……	300	300	Polymorphic
Band9	230	…….	……	230	230	……	Polymorphic
Total	9	6	6	7	8	7	……

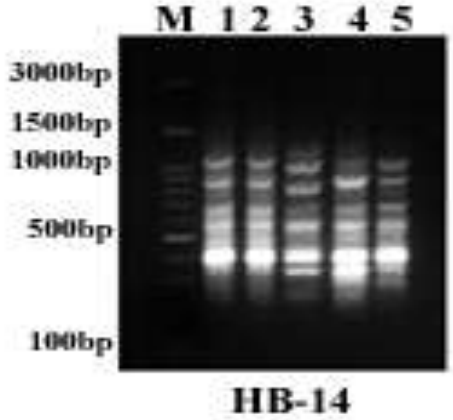

Fig. (4): RAPD-PCR produced for different treatments of *P. gossypilla* larvae using primer HB-14. Where ; lane 1=Control, lane 2 =Agristar, lane 3=Proclaim, lane 4= Tracer and lane 5= Camkron.

Data recorded in Table (9), the similarity index values ranged between 0.38 and 1.00 among the different tested larvae indicating high resistance in the treated 4th instar larvae to the toxicity of proclaim.

Table (9): Estimated similarity index between treated and untreated *P. gossypiella* larvae using primer HB-14.

	Control	Agristar	Proclaim	Tracer	Camkron
Control	-----	1.00	0.38	0.86	0.92
Agristar	-----	-----	0.77	0.86	0.92
Proclaim	-----	-----	-----	0.80	0.86
Tracer	-----	-----	-----	-----	0.93
Camkron	-----	-----	-----	-----	-----

According to the results which recorded in Table (10) and shown in Fig. (5), the primer A14 created 8 number of fragments, these fragments were 7, 5, 8, 8 and 8 associated with untreated larvae, agristar exposed larvae and tracer and Camkron-exposed larvae, each in that order. It was found that there were three polymorphic fingerprints and five monomorphic fingerprints for the five strains investigated. Untreated larvae and larvae treated with proclaim, tracer, and Camkron shared two pieces of 1270 and 540 pb. Only in larvae treated with proclaim, tracer, and Camkron did a 460 pb fragment show up. It was identified in all the larval treatments as well as in the untreated larvae.

Table (10): Total number and size of RAPD-PCR fragments generated by arbitrary primers in different treatments of *P. gossypiella* larvae using primer A-14.

MW-bp	**MW**	**Control**	**Agristar**	**Proclaim**	**Tracer**	**Camkron**	**Polymorphism**
Band1	1270	1270	…….	1270	1270	1270	Polymorphic
Band2	860	860	860	860	860	860	Monomorphic
Band3	540	540	…….	540	540	540	Polymorphic
Band4	460	…….	……	460	460	460	Polymorphic
Band5	400	400	400	400.	400	400	Monomorphic
Band6	320	320	320	320	320	320	Monomorphic
Band7	220	220	220	220	220	220	Monomorphic
Band8	180	180	180	180	180	180	Monomorphic
Total	8	7	5	8	8	8	……

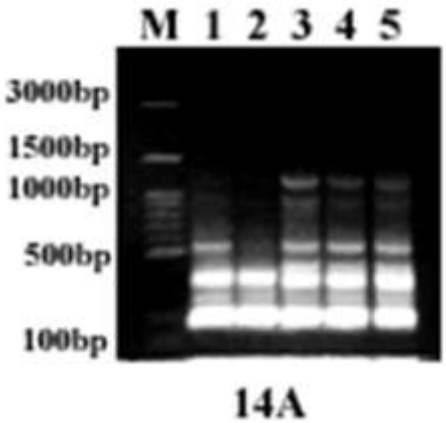

Fig.(5): RAPD-PCR produced for different strain of *P. gossypilla* larvae using primer A-14. Where is lane 1=Control, lane 2 =Agristar, lane 3=Proclaim, lane 4= Tracer and lane 5= Camkron.

Figures showing how closely *P. gossypiells* strains are related to each other using primerA-14 are shown in Table 1. (11). The collected results showed that the estimated similarity index between untreated and treated larvae varied from 0.77 to 1.00, which indicate sufficient tolerance to insecticide toxicity in the four field colony strains.. A substantial difference in insecticidal toxicity against pink bollworm larvae was found in the estimated similarity index, which varied from 0.77 to 1.00 for the four pesticides tested.

Table (11): Estimated similarity index between *P. gossypiella* larvae after different treatments using primer A-14.

Treatments	Control	Agristar	Proclaim	Tracer	Camkron
Control	-----	0.83	0.93	0.93	0.93
Agristar	-----	-----	0.77	0.77	0.77
Proclaim	-----	-----	-----	1.00	1.00
Tracer	-----	-----	-----	-----	1.00
Camkron	-----	-----	-----	-----	-----

Based on the aforementioned findings, it is reasonable to assume that the banding patterns were generated using random sequence short oligonucleotide primers (10bp in length) in the RAPD approach. In order to screen the whole genome for differences between two or more genomes under comparison, these arbitrary sequences are not exclusive to any one gene or DNA sequence. Homologous sequences can only be amplified if the opposing primer sites are about bp apart. Stress (e.g. pesticide treatment or variable temperature) may alter the base sequence of a primer binding site, which enables polymorphism to be identified in a population of organisms (Williams *et al*., 1990). It is possible that the term "random" in the acronym RAPD is deceptive since the sole random component is not the area amplified but the sequence of a primer. There are several benefits to using the RAPD-PCR approach to explore genetic variation. It's possible to sum up these benefits thusly: with no previous information of the DNA sequences for the genes of new species, it aids in genetic analysis. While biochemical (isozymes and proteins) markers are confined to a few genomic locations. This technique may be utilised for investigating mutations across populations exposed to any mutagen. The pace of evolution of various regions of the genome might vary greatly (Nei, 1987). PCR-amplified areas may thus develop at a faster pace. This procedure permits the simultaneous examination of several primers in a single run due to the minimal quantity of template needed in each reaction. The RAPD-PCR method does not, on the other hand, need the use of radioactive nucleotides, which is both costly and risky (Cenis and Beitia, 1994). An overnight PCR cycle may be started and the resulting products placed onto a gel to be examined the next day (Yoder *et al*., 1999). An oligonucleotide primer amplifies specific DNA segments in RAPD finger printing. RAPD markers are the name given to these DNA segments. The complementarity of a certain primer and template DNA sequence in a person determines the quantity and size of RAPD markers (Williams *et al*., 1993). Reproducible RAPD-PCR findings need high-quality DNA, as shown in the current study. In *P. gossypiell* larvae, the largest number of amplified fragments was 25, while the lowest number of amplified fragments was 14 resulting from primer C2. Primers don't all amplify in the same manner. According to Kantanen *et al*. (1995), some primers fail to amplify, while others yield banding patterns that are too much complicated. Using five primers of random sequences, the genomic DNA of the laboratory strain and the field colony strains were examined for signs of DNA damage or alterations in the sequence of genes. Changes in DNA sequence may be to blame for a RAPD pattern that doesn't include one particular bug. Results of RAPD analysis of *P. gossypiella* DNA from laboratory and field colony strains showed the lowest value of similarity index (0.0) which indicates the greatest degree of DNA structure and sequence variation between the genomes of the various strain using primer E7. Findings of the present study are in line with those of Abdel-Baset (2009) who reported result of modifications in the primer sequences for *P. gossypiella* and *Culex pipiens*, she discovered that the powerful tools OPA-13, OPA-15, and OPD-5 may be used to explore the genomic DNA alterations. For DNA or polymorphism screening, it is recognised that the sequence of the primer is the most important component. According to Salem (2018), the RAPD patterns generated by amplification of DNA structure and sequence differed between the genomes of untreated pink bollworm and those subjected to a broad range of various pesticides used to manage the pest in the field.

Abbreviations

PCR = polymerase chain reaction

RABD = Random amplied polymorphic DNA

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