Al Azhar Buletin of Science Vol.(25) No.2, December, 11-20, 2014,.

POPULATION STRUCTURE OF THE POTATO TUBER MOTH ,*PHTHORIMAEA OPERCULELLA* (ZELLER) , LEPIDOPTERA: GELECHIIDAE) IN DELTA EGYPT USING RAPD ANALYSIS.

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ABSTRACT

*Little is known about the nature and extent of genetic diversity in potato tuber moth Phthorimaea operculella (Zeller), Lepidoptera: Gelechiidae)***.** *Analysis of amplified fragment length polymorphism (AFLP) has the potential to become a powerful new DNA fingerprinting technique for studying genetic relationships and genetic diversity in insects. Random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR)was used to examine variation in three governorates in the Delta of Egypt. The objective of this study was to provide baseline information on the population structure of potato tuber worm in Egypt. The applicability of CTAB -based DNA extraction protocol provided suitable for AFLP analysis. Potato tuber worm adults were collected from potato fields in the major production regions in the Egypt . We used 20 random 10-oligomer primer individuals and 179 polymorphic AFLP bands were used to infer the geographic population structure of potato tuber worm. This study provides baseline data for the molecular characterization of potato tuber worm populations, which will aid in tracking the origin of future invasions within Egypt.*

Keywords*: DNA extraction, amplified fragment length polymorphism, insects*

INTRODUCTION .

Potato tuber moth (PTM), *Phthorimaea operculella* (Lepidoptera: Gelechiidae) Zeller is a widely distributed, devastating pest of potatoes (*Solanum tuberosum* L. in the tropics and subtropics) attacking the foliage and infest the tubers in both field and store causing serious economic damage (Sudeep *et.al* .,2005). Larvae damage potatoes by tunneling tubers and mining leaves, stems, and petioles when in the larval stage. The patterns of population increase and range of expansion of this pest suggest that potato tuberworm populations are overwintering in this area rather than recolonizing or being reintroduced from a moderate latitude. There is no information regarding how or when exactly potato tuberworm arrived in the Egypt. The only piece of information regarding this issue is the records of potato tuber worm collections in California (Chittenden 1913, Radcliffe 1982, Jensen *et al.* 2005, Rondon *et al*. 2007, Rondon 2010). The analysis of genetic variation using DNA fingerprinting techniques has become an important approach in taxonomic , population genetic and evolutionary studies of a variety of insect species .The most frequently used DNA markers include restriction fragment length polymorphisms (RFLPs) of mitochondrial or nuclear DNA ,DNA fingerprinting of microsatellite or minisatellite sequences ,standard polymerase chain reaction (PCR) and random amplified polymorphic DNA (RAPD)analysis of molecular technique nuclear .A diverse array of molecular technique available for high –resolution genetic studies of population level processes .Random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) using a single primer ,simultaneously amplifies many regions of genomic DNA (Williams *et al* ., 1990). RAPD is being used as a reliable technique for distinguishing insect. Amplified fragment length polymorphisms (AFLP) were used to determine the genetic population structure of potato tuberworm within the United States. (Medina *.et.al*.2010) Genetic population structure is often observed in insects with a wide geographic distribution (Roderick 1996, Mynhardt *et al*. 2007). Information on a pest's genetic population structure is important because it provides insights into levels of gene flow across a pest's geographic distribution. Genetic characterization of pest populations across their geographic distribution is needed to generate baseline information that may allow us to pinpoint the origin of future pest introductions. Thus, the objective of this study was to determine the population structure of potato tuberworm in the Delta Egypt to provide such baseline information.

MATERIAL AND METHODS

Insects collected in three governorates Dakahlia ,Gharbia and Behaira (i.e . , from the three different locations) The strains of the potato tuber moth *P.operculella* were reared in the laboratory as described by Fenemore (1977).) ,maintained at 25ºC and 12L:12D Photoperiod.

1. DNA extraction with CTAB method (modified from Sambrook and Russell 2001) Place each 80 mg of (frozen or equilibrated) (larva or pupa,or adult) in a 1.5 ml Eppendorf cup (without fluid), put label (each site or governorates) on the cup, put cups on ice.

Put fluid nitrogen in each Eppendorf cup, quickly close the lid for a short time (for preventing the sample to "jump out"), and immediately crush animals with clean pestles; then add 500 µl of warmed (65 °C) 1% (or 2%) CTAB solution. Incubate the mixture for 1 h at 65 °C in the Thermomixer. Let the mixture cool down to 55 °C (and set the Thermomixer to 55 °C), then add 2 µl-10µl Proteinase K and incubate in the Thermomixer for 3-4 h at 55 °C or overnight (old animals always over night). Add 500 µl Chloroform / Isoamyl alcohol, vortex, and centrifuge for 5 min at 14000 rpm (rounds per minute) (RT). After centrifugation put upper phase of each sample in a fresh cup (if individuals are very old or dirty, begin this procedure again from the start). Be cautious not to get the tiniest part of the lower phase mixed with the upper phase. Add 40 µl NaAc (1/10 Vol., 3 M, ph 4.8)mix and 350 µl Isopropanol (-20 °C), shortly shake the cup, then incubate 30 min at -20 °C or overnight (old animals at least 1 h) in the freezer; if not over night ➔cool down centrifuge in time

Centrifuge for 30 min at 4 °C and 14000 rpm; discard supernatant carefully with pipit.

Place the cups on ice or in a cooled rack; wash** each pellet with 300 µl 100% EtOH (p.a.) (-20 °C), then centrifuge 10 min at 4 °C and 14000 rpm; discard supernatant carefully with pipit. Place the cups on ice or in a cooled rack; wash** pellet with 300 µl 70% EtOH (p.a.) (-20 °C), then centrifuge 5 min at 4 °C and 14000 rpm; discard supernatant carefully with pipit.

Wash** pellet with 150 µl 70% EtOH (p.a.) (RT), then centrifuge 5 min at RT and 14000 rpm; discard supernatant carefully with pipit. Dry DNA pellet in open Eppendorf cup for 2 min at 50 °C (in the Thermomixer) or for at least 10 min (better 30 min) at RT (in the flue . Resolve the pellet in PCR water or TE buffer (pH8): Give enough time for dissolving (e.g. 1 h in the fridge.

PCR reaction mixtures consisted of 1µl DNA, 2 pmol dNTPs, 0.5 pmol of each primer, 9µl dd H2O, 2µl 10x PCR buffer (without MgCl2), 2 mM MgCl2, and 0.4µl of 1 unit/µl Taq Polymerase (Q Bio Gene). PCR-Programm: (for all the same 1) 1 :94°C pause 2 :94°C 4 min 3:94°C 1 min 15 sec 4: 54°C 45 sec 5: 72°C 25 sec 33 cycles ; back to step Nr. 3 6:72°C 1min 7:10°C pause. PCR–products were checked on 1.5% agarose gels stained with ethidium bromide.

1 .Similarity coefficient Nei and Li coefficient (1979):

$$S = 1 - \frac{N_{ab}}{N_a + N_b}$$

Where: N a b : the number of common bands in the two samples "a" and sample "b"

Na: the number of bands in sample "a" Nb: the number of bands in sample "b"

2. Commonality or sharing band ratio: (**Haymer and Mc Inns**,1994)

Commonality % =

No. of common bands in samples A and B

……..x 100

No. of total bands of both samples A and B

3.Genetic distance.

Genetic distance = 1 - S

where S.co: Similarity coefficient

4.Cluster analysis.

The presence /absence of band was analyzed by Cluster analysis and principal Coordination Analysis (P.C.A) by using the MVSP version 3.10b (1985-1999) Kovach computing services.

Table(1) The nucleotide sequences of primers used for RAPD-PCR .

NO	primers	Sequence5,to 3”	NO	primers	Sequence5,to 3”
1-	Pr1	CCC AAG GTC C	11-	Pr11	GAG TCT CAG G
2-	Pr2	GGT GCG GGA A	12-	Pr12	TTA TCC CCC C
3-	Pr3	CCA GAT GCA C	13-	Pr13	CCC GAT TCG G
4-	Pr4	GTG ACA TGC C	14-	Pr14	TGC GGC TGA G
5-	Pr5	TCA GGG AGG T	15-	Pr15	ACG CAC AAC C
6-	Pr6	AAG ACC CCT C	16-	Pr16	GGT GAC TGT G
7-	Pr7	AGA TGC AGC C	17-	Pr17	CTA CTG CCG T
8-	Pr8	TCA CCA CGG T	18-	Pr18	GGA CTG CAG A
9-	Pr9	CTT CAC CCG A	19-	Pr19	ACG GCG TAT G
10-	Pr10	CAC CAG GTG A	20-	Pr20	AAC GGT GAC C

RESULTS

After excluding bands that were not clearly identifiable that were monomorphic for the entire data set, a total of 179 polymorphic bands were scored from 20 primers .The number of polymorphic marker bands ranged from one to eight, depending on the primer. RAPD

divergence among regions (Beheira, Gharbia and Dakahlia) were attributable to bands exhibiting variable frequencies rather than to bands exhibiting fixed differences. Several bands provided good differentiation between regions (Beheira, Gharbia and Dakahlia) populations for example Pr1 in Beheira governorate found 3 bands and in Dakahlia governorate but absent in Gharbia governorate ,The fragments had size range from 699 to 417bp,it was noted that samples of Beheira and Dakahlia produced genomic fingerprints patterns sharing three bands .This was subsequent by the similarity coefficient, . Commonality percentage and genetic distance according to data presented in Table (3).Also observed Pr2 in Beheira governorate were 8 bands and also 8 bands in Gharbia governorate but single band in Dakahlia governorate .The amplified fragments had approximately size range 716 to 268 bp , moreover showing one common band 533bp among all populations under investigation ,samples of Beheira and Gharbia produced genomic fingerprints sharing bands .The similarity coefficient ,commonality percentage and genetic distance are presented in Tables (3) . Pr3 scored 3 bands in Beheira governorate, 4 bands in Gharbia governorate and 6 bands in Dakahlia governorate .The fragments had size range 771 to 326 bp.samples of Beheira and Gharbia sharing 3bands , Beheira and Dakahlia 3bands ,and also Gharbia and Dakahlia 4bands The similarity coefficient ,commonality percentage and genetic distance are presented in Tables (3) and Fig (1) . Pr4 showed 5 band in three populations size range 374 to 190 bp , it was noted that ,samples produced genomic fingerprints sharing the all bands . The similarity coefficient ,commonality percentage and genetic distance presented in Tables (3) and Fig(1). Pr5 found absent in Beheira governorate, single bands in Gharbia governorate and 4 bands in Dakahlia governorate size ranged 374 to 186 bp , where one band only with molecular size 186 bp was common among their patterns. . Pr6 observed 4 bands in Beheira governorate, absent bands in Gharbia governorate and 2 bands in Dakahlia governorate. The amplified fragments had approximately sized from 351 to 198 bp .RAPD patterns produced by using Pr-6 were characterized by the presence of only one common band . Pr7was obvious that

a total of 8 reproducible fragments appeared in three region tested of Potato tuber moth attributed single band in Beheira governorate, absent in Gharbia governorate and 7 bands in Dakahlia governorate .The fragments had approximately sized from 632 to 233 bp and one common band ,its molecular size 466 bp. Pr8 was obvious that a total of 4 reproducible fragments appeared in all tested Potato tuber moth , Beheira and Gharbia had one common band sharing with them, Dakahlia samples of Potato tuber moth had two bands 367 and 260bp different molecular size which did not share any bands, The similarity coefficient ,commonality percentage and genetic distance presented in Tables (3) and Fig(1). Pr9 found one band in Dakahlia with molecular size 618bp ,in Beheira 3bands and Gharbia 7 bands had molecular size ranged 618 to 163 bp .

The RAPD- PCR pattern of Beheira, Gharbia and Dakahlia were characterized by one common band ,but between Beheira and Gharbia had 3 common fragments with molecular sizes 468,385 and 225 bp . Pr10 amplified fragments were not detected in the three samples under study. Pr11examination of characteristic patterns of RAPD-PCR revealed 7 reproducible fragments of sample of Dakahlia only but Gharbia and Beheira did not produce fragments.Pr12,it was obvious that the tested samples of Potato tuber moth had 8 reproducible fragments of molecular size range from 769 to 278 bp ,Beheira ,Gharbia and Dakahlia shared single band with molecular size 307 bp . The similarity coefficient , commonality percentage and genetic distance presented in Tables (3) and Fig(1).The amplified fragments of genomic of fingerprints Pr13 had molecular sizes ranged from 980 to 79 bp ,total number of reproducible fragments detected in tested samples were 14 fragments, RAPD-PCR patterns of Potato tuber moth samples of Beheira and Gharbia were characterized by having four common fragments with molecular size 737,635,506,and 342 bp. RAPD-PCR patterns of Beheria and Dakahlia were characterized by two common bands with molecular size as 635and 506 bp in three samples with tested.Pr14 was obvious that the tested samples of Potato tuber moth had two common bands with molecular sizes 747 and 695 bp .moreover, a total of 11reproducible fragments appeared in all tested samples .The amplified fragments had molecular size ranged from 992 to 380 bp. Pr 15 examination of characteristic patterns of RAPD-PCR revealed 15 reproducible fragments of samples of 8 Dakahlia 4 Gharbia and 3 Beheira ,and also having three common fragments.Pr16,it was obvious that the tested samples of Potato tuber moth had 14 reproducible fragments, Beheira have 5 bands , Gharbia having 4

bands and Dakahlia having 5 bands. Morever Beheira and Gharbia sharing 3 bands molecular size from 581,481 and 286 bp ,Beheira sharing Dakahlia 2 band and also Gharbia sharing Dakahlia 2 bands with molecular size716 and 539 bp The similarity coefficient , commonality percentage and genetic distance presented in Tables (3) and Fig(1).Pr17 examination of characteristic patterns of RAPD-PCR revealed 9 reproducible fragments of samples of Beheira and Dakahlia but Gharbia did not produce fragments, Beheira and Dakahlia having 2 common bands with molecular size1049 and 653 bp.Pr18 examination of characteristic patterns of RAPD-PCR revealed 4 reproducible fragments of sample of Dakahlia only but Gharbia and Beheira did not produce fragments. Pr19 revaled11 reproducible fragments except Gharbia was not reproducible fragments .The fragments had approximately sized from903 to 378 bp ,Samples of Beheira and Dakahlia produced genomic fingerprint patterns sharing the five bands.Pr20 examination of characteristic patterns of RAPD-PCR tested three samples Potato tuber moth ,revaled four reproducible fragments of one only sample of Dakahlia.

The total number of RAPD-PCR fragments generated by a battery of 20 primers in Beheira governorate were 66 bands ,all primers reproducible fragments except Pr5,Pr10,Pr11and Pr20 .And the number of RAPD-PCR fragments generated by a battery of 20 primers in Gharbia governorate were 44 bands, all primers reproducible except Pr1, Pr6, Pr7 Pr10, Pr17, Pr18, Pr19 and Pr20. Also of RAPD-PCR fragments generated by a battery of 20 primers in Dakahlia governorate were 73 bands all primers reproducible except Pr10.The characteristic patterns of ampli-

fied PCR –bands were illustrated in Fig(1) and Table (2).

Measurement of genetic similarity and genetic distance among the three Potato tuber moth samples collected from different governorates:

The genetic similarity between populations representative of the different RAPD types was calculated and shown in the form of Dendrogram in Table(3) and Fig(2).

All the tested Potato tuber moth populations are grouped into two main clusters .The first cluster included Potato tuber moth populations collected from Dakahlia governorates. On the other hand ,the second cluster was divided into two populations .the first population included Potato tuber moth populations of Gharbia governorates and second populations Beheira governorates

Tree diagram for 3 variables complete linkage Euclidean distance

Fig(1):Nei and Li's coefficient of the relationship (Dendrogram) among Potato tuber moth collected from three governorates (Beheira,Gharbia and Dakahlia).

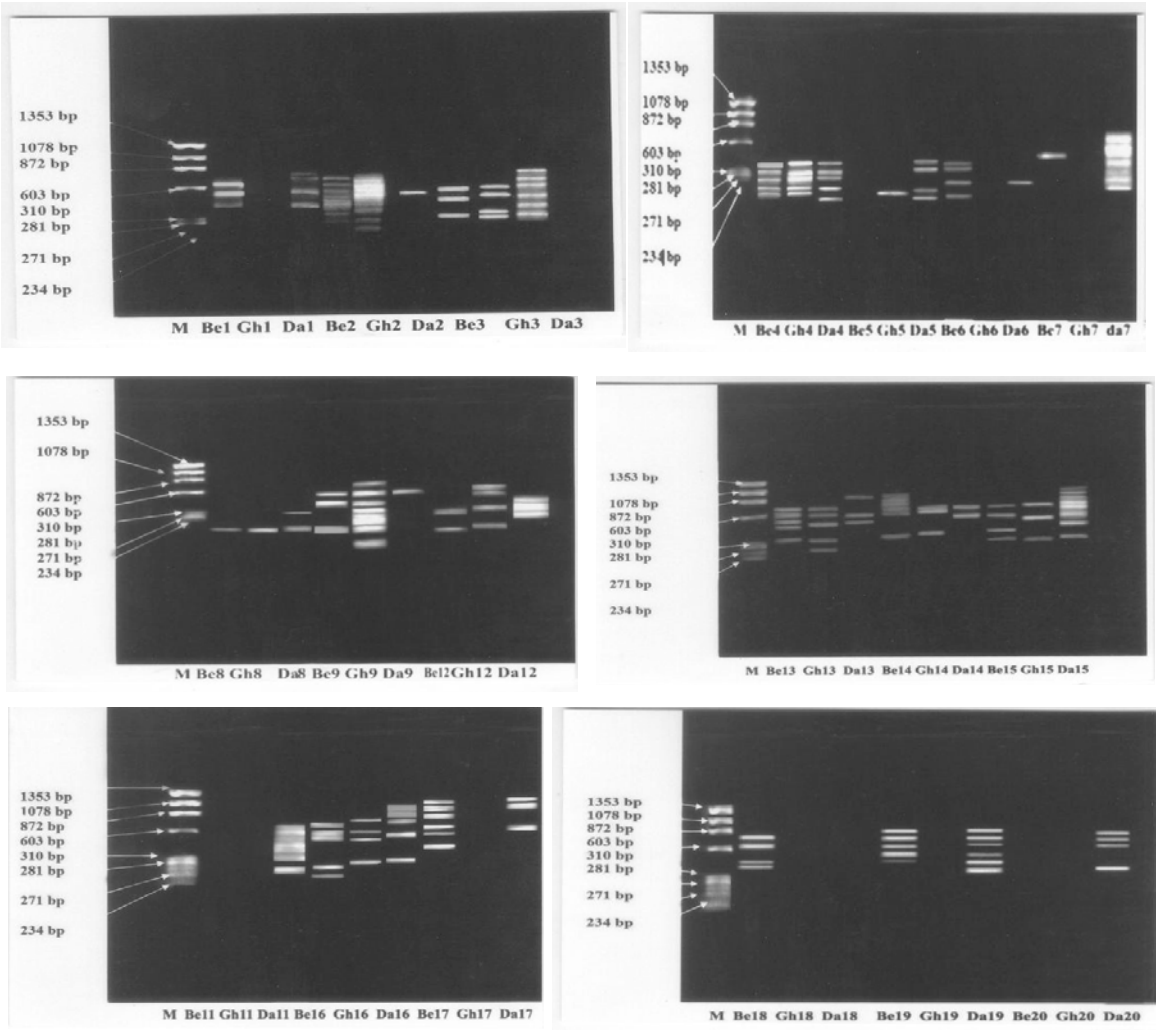

Fig (2) RAPD-PCR Patterns of three samples of Potato tuber moths collected from three different governorates using 20 primers on Beheira (Be),Gharbia (Gh) and Dakahlia(Da) respectively .Lane M represents the molecular size marker .DNA sample were separated on 1.8% agarose gel and stained with ethidium bromide.

Table(2) : The total number of RAPD-PCR fragments generated by a battery of 20 primers in the three samples of Potato tuber moth collected from different governorates

Primers	Beheira	Gharbia	Dakahlia	Total no of DNA fragments
Pr1	3	-	3	6
Pr2	8	8	1	17
Pr3	3	4	6	8
Pr4	5	5	5	15
Pr5	-	1	4	5
Pr6	4	-	1	6
Pr7	1	-	6	7
Pr8	1	1	2	4
Pr9	3	7	1	11
Pr10	-	-	-	-
Pr11	-	-	6	7
Pr12	2	4	3	9
Pr13	5	5	3	13
Pr14	6	3	2	11
Pr15	4	3	8	15
Pr16	5	4	5	14
Pr17	7	-	3	10
Pr18	4	-	-	4
Pr19	5	-	6	11
Pr20	-	-	4	4
	65	45	69	179

DISCUSSION

The use of random amplified polymorphic DNAs (RAPDs) along with microsatellites ,and new DNA analysis techniques have recently become quite popular .These methods employ an approach can be referred to as the blind analysis of DNA. RAPD-PCR technique is very important development because it means that genetic analysis can be applied on a new species without any prior knowledge about the particular DNA sequences or the genes from this new species. RAPD-PCR offers several advantages over other methods used for studying genetic variability among populations because it is not limited to a single locus but theoretically detects polymorphisms across the whole genome either coding or non coding regions ,in contradiction to isozyme markers that are limited to a few genomic-regions ,so that this method may be used for studying mutation among populations exposed to any mutagen (such as chemical substance, pesticides and radiation).Different parts of the genome can evolve at different rates (Nei,1987). Therefore, it is possible to show one or several regions amplified by PCR evolving at a higher

Table (3) :Similarity coefficient,commonality percentage and genetic distance among three samples of Potato tuber moth collected from three different governorates of Delta Egypt using 20 primers.

	Similarity coefficients			Commonlity%			Genetic distance		
	Be/Gh	Be/Dak	Gh/Dak	Be/Gh	Be/Dak	Gh/Dak	Be/Gh	Be/Dak	Gh/Dak
1	-	0.57	-	-	42.9	-	-	0.43	-
2	o.87	0.89	0.56	43.8	11.11	11.11	0.11	0.11	0.56
3	0.57	0.67	0.6	42.9	33.33	40	0.43	0.33	0.4
4	0.5	0.5	o.5	50	50	50	0.5	0.5	0.5
5	-	-	0.8	-	-	20	-	-	0.2
6	-	0.8	-	-	20	-	-	0.2	-
7	-	0.8	-	-	20	-	-	-	0.2
8	0.5	-	-	50	-	-	0.5	-	-
9	0.70	0.75	0.88	30	25	12.5	0.3	0.25	0.12
10	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-
12	0.8	0.8	0.83	20	20	16.7	0.2	0.2	0.17
13	0.6	0.77	0.77	40	22.22	32.22	0.4	0.23	0.23
14	0.77	0.75	0.6	22.22	25	40	0.23	0.25	0.4
15	0.88	0.69	0.73	12.5	30.77	27.3	0.12	0.31	0.23
16	0.66	0.8	0.77	33.3	20	22.22	0.34	0.2	0.23
17	-	0.77	-	-	22.22	-	-	0.23	-
18	-	-	-	-	-	-	-	-	-
19	-	0.54	-	-	45.45	-	-	0.46	-
20	-	-	-	-	-	-	-	-	-

rate compared with the enzymatic loci. Thus a higher variability can be obtained by RAPD-fingerprints specially if the amplified DNA regions contain micro and or minisatellites (Baruffi *et al*., 1995).The extensive polymorphisms detected among individuals indicate that RAPD-PCR can probably be used in a variety of ways for some species to measure clone diversity among populations and determine the degree of relatedness among individuals and races .In Random Amplified Polymorphic DNA(RAPDs),banding profiles are created using small oligonuc DNA(RAPDs),banding profiles are created using small oligonucleotide primers (around 10 bp in length)of arbitrary sequence. The profile generated by the RAPD typing system are independent of the gene expression and not influenced by ontogenetic and environmental factors .The sensitivity of the RAPD-PCR technique was higher than the other methods of fingerprinting both in terms of the samples quantity and quality .In RAPD fingerprinting, an oligonucleotide primer amplifies distinct DNA fragments. These fragments are referred to as RAPD markers. The number and size of RAPD markers depend on the complementary sequence of particular primer and template DNA(Hedrick,1992 and Williams *et al* .,1990) .Numerous factors can affect the reproduce and standardization of reactions as DNA quality and quantity ,Mg^{2} + or concentratrations and Taq polymerase source (Loxdal and lushhai 1998).In taxonomy and systemic ,species –specific RAPD markers could be an invaluable tool for species verification and establishing the status of organisms of controversial systematcs .Moreover ,RAPD-PCR provided an additional opportunity to judge the relative merits of isozyme and RFLP analysis. In the present study, RAPD-PCR c yield reliable and useful results, if certain points were considered .The risk of misinterpretation in RAPD analysis if different RAPD fragment have similar size ,can be minimized by the use of several RAPD markers and the reproducibility of these markers .The homology of RAPD fragments, optimization of reaction components and condition of RAPD-PCR is essential to have reproducible results .The optimization process included the use of a specific combination of template DNA and primer, the use of the standard conditions for extraction and amplification of DNA as well as the extreme care in sterilized of buffers ,tubes and tips .The same finding were reported by (Virk *et al* ., 1995 and Dinesh *et al* 1993). RAPD-PCR in potato tuber moth ,*phthorimaea operculella* populations as in the other insects produced a serious of discrete DNA fragments which typically vary in intensity and ranged in size from 118bp larger than 1350bp.In addition to several reported factors affecting reproducibility of RAPD amplification ,this study has established that different concentration of primer influence banding patterns .Genomic DNA of potato tuber moth ,*phthorimaea operculella,*produced more reliable banding patterns when the primers were used at concentration 20-25 pM . Virk *et al*., 1995) reported that the banding pattern of any genomic DNA depends mainly on the frequency of annealing sites for the primers used and on the effective concentration of such primers in the reaction tube .The number and size of amplification products depend on the complementarity of sequence of particular primer and template DNA .The present results also agree with what has been reported by (Bardakci and Skibinski 1994) ,who found that broad agreement across primers but overall level of similarity was varied between primers .Therefore ,the choice of primers is of a major importance for the discriminatory power of the technique. (Foster *et al*., 2004., Duan *et al*.,2004 and Kim and Sappington .,2004) analyzed RAPD-PCR pattern and genetic differentiation among boll weevil populations from eighteen locations across eight US states and North-East Mexico. Sixty –seven reproducible bands from six random primers were analyzed for genetic variation within and between boll weevil population . Genetic and geographic distances among all populations were positively correlated, reflecting a pattern of isolation by distance within a larger population .Gene flow between, South-Central ,Western and Eastern regions is limited, but migration between locations within regions appears to be relatively frequent up to distances 300-400Km.In any species ,the RAPD technique amplifies some regions of DNA that are monomorphic but it simultaneously produce other bands that are identified as

unique set of genotypes in each species. There are several explanations for the variability detected by this method. It is possible that variation is due to the presence of primer binding site in highly regions .(Sudeep *et al*., 2005 and Lery *et al* .,2003) analyzed the RAPD-PCR was used to characterize 11 insect cell lines including six from Lepidoptera (five species) one from dipteral and four from coleopteran (one species : *Leptinotarse decemlineata*) .whatever the order and even when comparing two closely related species from the same genus (*Spodoptera*),the DNA fingerprints are different from one species or from one primer to the another .On the other hand ,two independently isolated cell lines from the lepidopteran *Phthorimaea operculella* produce nearly identical profile with only minor differences .Finally ,a statistical analysis based on Nei□ s similarity coefficient .Each possesses a common recognizable pattern was found in filed collected. While showing also a series of a polymorphic markers which allow one to distinguish each cell line from the three other RAPD Fingerprinting . The present results reveal that RAPD are effective markers in determining polymorphism and estimating the variability and genetic distance between the studied potato tuber moth samples .The differential amplification may be due to sequence difference in the priming sites (Williams *et al*., 1990 and Sultmann .,1995) or due to point mutation ,which allow or abolish primer binding (Hedrick ,1992) .Thus the polymorphisms are essentially due to differences in frequency and distribution of priming sites .However ,the specific interpretation of polymorphisms obtain by RAPD technique is yet open to discussion. Our results are harmony with this trend, where in the present study three populations from different governorates were tested .All the tested populations are grouped into main clusters. The first cluster included potato tuber moth populations from Beheira and Gharbia .The second grouped included potato tuber moth populations of Dakahlia governorates. The above conclusion was conducted from cluster analysis and Principal Coordination Analysis (P.C.A) to verify that the results did not depend on type of statistical analysis used statistical data of P.C.A confirmed that the cluster analysis. RAPD have provided new and useful information, which will assist further research on this important agricultural pest .The results presented here demonstrate the use of the RAPD technique ,which may have application in studies of other populations.

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