



# GENETIC DIVERSITY ASSESSMENT OF SOME STORED INSECT SPECIES IN IRAQ BASED ON RAPD MOLECULAR MARKER

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## Abstract

Genotyping of four insect species was carried out using RAPD marker. The genetic variability among the four insect species was estimated using RAPD primers. All primers generated reproducible and easily storable RAPD profiles with some amplified DNA fragments ranging from 9 to 13. The total number of amplicons detected was 56, including unique bands, reached seven, Number of polymorphism was 49 and this represents a level of polymorphism of 87.04% and an average number of 6.8 polymorphic bands per primer. Maximum numbers of amplicons were amplified by primer U-17 reached 13 while the minimum number of fragments was amplified with primers OPE K-02 reached nine. The highest number of polymorphic bands reached 12 were obtained with primer U-17, while the highest number of monomorphic bands reached two was obtained with primer OPE K-02, and M-32 with percentage reached 22.22% and 16.664, respectively. RAPD markers detected genetic similarity and distance, a maximum genetic distance value was observed between *T. granarium*(1) and *C. maculatus*(4) and *S. oryzae*(2) and *C. maculatus*(4) species reached 0.547 with less similarity value reached 46%, a minimum genetic distance value was 0.435, which observed between *S. oryzae* (2) and *T. castaneum* (3) with high similarity value reached 57%. The similarity matrices were employed in the cluster analysis to generate a dendrogram using the UPGMA method. The cluster tree analysis showed that the insect species were broadly divided into two main groups A and B with genetic similarity reached 30%. A group including *T. granarium* (1) B group was divided into two sub-cluster B1, and B2 with genetic similarity reached 36%. The first sub-cluster (B1) including only *C. maculatus* (4) while the second sub-cluster (B2) included two species *S. oryzae* (2) and *T. castaneum* (3) with the high genetic similarity between them reached 48%.

**Key words :** Genetic diversity, molecular marker, RAPD, store product insects, plant protection.

## Introduction

Stored grain products are one of the largest sources of food in the world (Alonso-Amelot and Avila-Núñez, 2011). The evidence has shown that economic losses caused by insects infested stored grain and insects contamination on food are increasing (Riudavets *et al*, 2010; Wei, 2012). Recent investigations indicated that insect poses a new risk to global food security (Athanasios *et al.*, 2009). In this study, the Insectes were selected are *Tribolium castaneum* (Family: Tenebrionidae), *Trogoderma granarium* (Family: Dermistidae), *Callosobruchus maculatus* (Family: Bruchidae) and *Sitophilus oryzae* (Family:

Curculionidae) they are considered as a very serious pest that caused economic loss that reached 20% in crops after harvest and may reach to 80% in some third world countries (Arthur, 2000).

Insects include the largest species composition in the entire animal Kingdom and have a vast undiscovered genetic and gene pool that can be better explored by using molecular marker techniques. DNA markers are used to provide raw information based on which an ecologist make estimates of genetic diversity and gene flow between species (Behura, 1999). Over the last 15 years, DNA markers had made a significant contribution to the rapid rise of molecular studies of phylogeny, genetic

relatedness and population dynamic (Avisé, 2004) and gene and genome mapping in insects (Severson *et al*, 2001; Heckel, 2003). In molecular markers, RAPD-PCR is a conceptually simple technique for estimation of genetic diversity of organism (Williams *et al*, 1990; Welsh and McClelland, 1990). RAPD markers have become the most common yardsticks for measuring genetic difference between individuals, within and between related species population. This study aimed to evaluate the genetic distance and similarity between four species of stored product insects by using five RAPD primers.

## Materials and Methods

### Insect collection and rearing

Insects were collected from different places in Najaf city. They were identified morphologically at University of Baghdad / Iraq Natural History Museum. It was *Tribolium castaneum*, *Trogoderma granarium*, *Callosobruchus maculatus* and *Sitophilus oryzae*. They were bred in a laboratory of insects, dept. of plant protection, agriculture faculty, University of Kufa under dry and dark conditions at  $28 \pm 2^\circ\text{C}$  temperature and  $60 \pm 5\%$  relative humidity. *T. castaneum* bred on wheat flour, while *C. maculatus* fed on white bean seeds and wheat seeds were used to breed *T. granarium*, as chick peas were suitable for (wheat). Random amplification polymorphic DNA (RAPD) used as an indicator to measure genetic covariance and genetic distance among insect species.

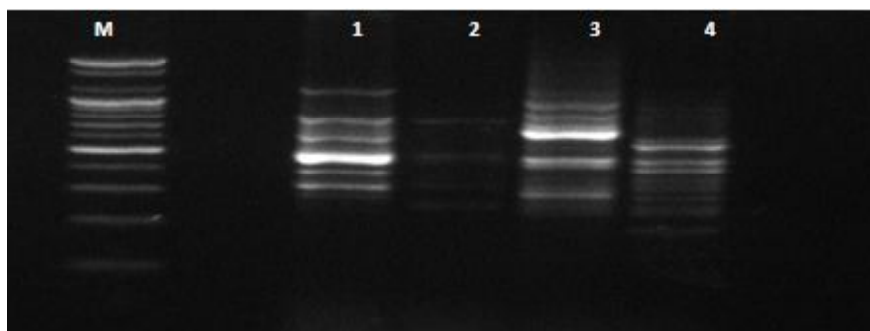
### DNA isolation

Total genomic DNA was isolated from adult insects' species using the Kit, leaf samples (50 mg) of fine powder were ground in liquid nitrogen. DNA was extracted by using Genomic DNA Mini Kit (Geneaid/UK). 200  $\mu\text{l}$  of DNA extracted was stored at  $-20^\circ\text{C}$  until use. Quantity, Concentration and quality of DNA were determined by Nano drop-spectrophotometrically at 260 nm. The study was accompanied in the research laboratory of Molecular Genetic at the University of Kufa, genetic engineering, and biotechnology laboratory.

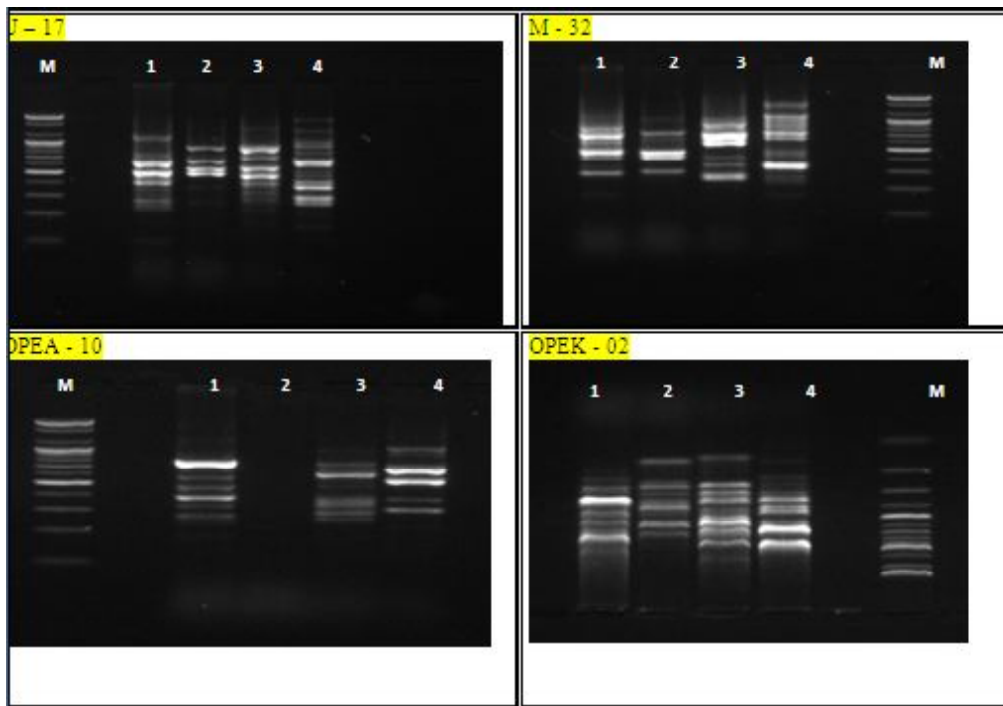
The RAPD primers (table 1) were obtained from BIONEER, South Korea. A total of 5 decamer oligonucleotides of arbitrary sequence were tested for PCR amplification. AccuPower Gold Multiplex PCR premix (BIONEER/ South Korea) was used to DNA amplification with RAPD primers and the thermal cycler conditions for PCR reactions were an initial denaturation cycle of 1 min and 30 s at  $94^\circ\text{C}$  was followed by 45 cycles comprising 1 min at  $94^\circ\text{C}$ , 1 min at  $36^\circ\text{C}$  and 2 min at  $72^\circ\text{C}$ . An additional cycle of 7 min at  $72^\circ\text{C}$  was

**Table 1 :** Total number of amplicons, polymorphic, monomorphic amplicons and percentage of monomorphism, polymorphism as revealed by RAPD markers among the 4 insects accessions.

Monomorphic Fragments Percentage (%)	Number of Monomorphic Fragments bands	Polymorphism Fragments Percentage (%)	Number of Polymorphic Fragments bands	Unique Fragments Bands Percentage(%)	Number of Unique Fragments bands	Number of Total amplified fragments	Primer sequences 5' to 3'	RAPD Primers
9.09	1	90.90	10	45.45	5	11	GGATGAGACC	OPE-G14
9.09	1	90.90	10	54.54	6	11	GTGATCGCAC	OPEA-10
22.22	2	77.77	7	22.22	2	9	GTCCTCCGCAA	OPEK-02
7.69	1	92.30	12	41.66	6	13	CTGCCAGCAT	U-17
16.66	2	83.33	10	42.00	5	12	GGGGCCTTAA	M-32
12.35	7	87.04	49		7	56	TOTAL	
	1.4		9.8		1.4	11.2	Average	



**Fig. 1 :** Isolated the total DNA of the *T. granarium* (1), *S. oryzae* (2), *T. castanum* (3) and *C. maculatus* (4) on an agarose gel (1.5%) and voltage (100 V) for (30 minutes).



**Fig. 2 :** RAPD profiles of the 4 insects genotypes amplified with RAPD primers, M: molecular weight marker *T. granarium* (1), *S. oryzae* (2), *T. castanum* (3) and *C. maculatus* (4) on an agarose gel (1.5%) and voltage (100 V) for (30 minutes).

used for final extension.

### Electrophoresis of DNA

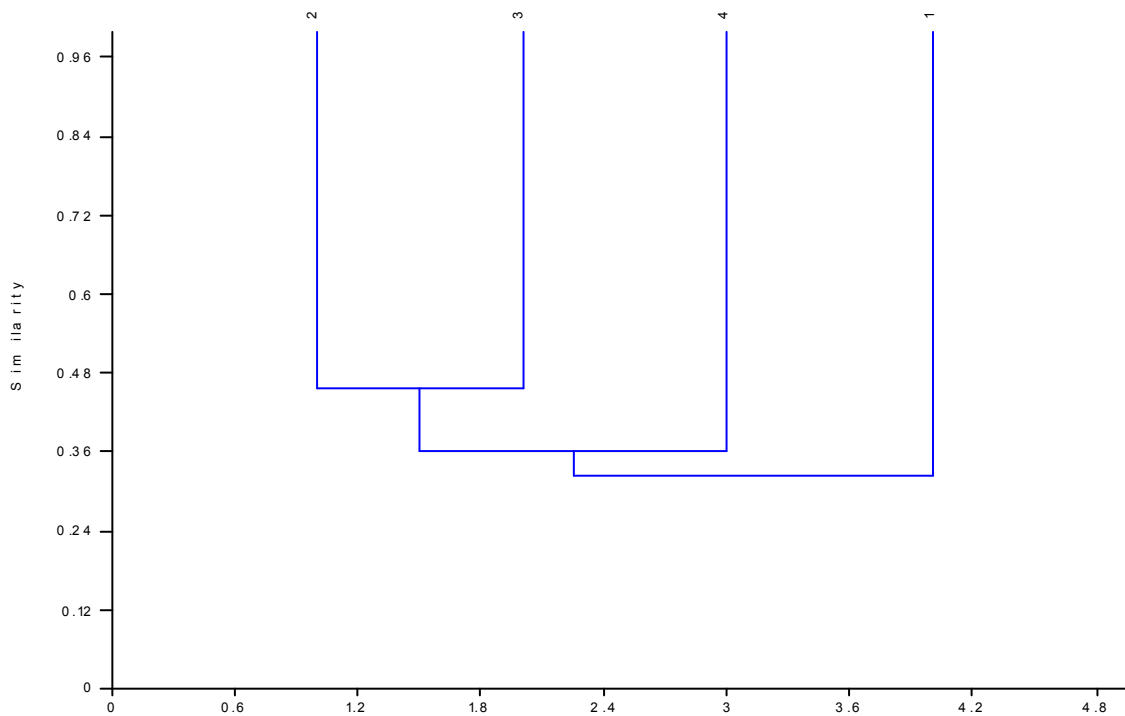
Amplification products were divided by electrophoresis (100 V) for (30 minutes) in 1.5% agarose gels and stained with ethidium bromide. A photographic record was occupied under UV illumination.

### Data analysis

Only repeatable and clear application products were scored as one for present bands and 0 for absent ones. The specific bands suitable for identifying species and cultivar were termed with primer number tracked by size for the approximate of the amplified fragment in pairs base. Amplified products were evaluated by pairwise - comparisons of the genotypes grounded on the percentage of a similarity matrix and common fragments was

calculated (Nei and Li, 1979). The 0 or 1 data matrix was created and used to calculate the genetic distance and similarity using 'Simqual,' a subprogram of the numerical taxonomy and multivariate analysis system program (NTSYS-PC) program (Stangl *et al.*, 2005). A dendrogram was created based on the genetic space matrix by applying an unweighted pair group technique with arithmetic averages (UPGMA) cluster analysis using the Molecular Evolutionary Genetics Analysis) MEGA version 2.0 (Tamura *et al.*, 2007).

The results in fig. 1 showed of isolated total DNA of the insects of the intentional olive cultivars means filters and then migrated to agar gel 1.5%, voltage 100 V for 30 minutes noting the achievement of the method to isolate DNA from this insect species.



**Fig. 3 :** Dendrogram for the 4 insect's genotypes constructed from RAPDs data using unweighted pair-group arithmetic Average (UPGMA) and similarity matrices computed according to coefficients *T. granarium* (1), *S. oryzae* (2), *T. castanum* (3) and *C. maculatus* (4).

**Table 2 :** Genetic distance among insect species *T. granarium* (1), *S. oryzae* (2), *T. castanum* (3) and *C. maculatus* (4).

4	3	2	1		
0.547		0.538	0.529	0	1
0.547		0.435	0	0.529	2
0.5		0	0.435	0.538	3
0		0.5	0.547	0.547	4

### Monomorphisms and polymorphisms detected by RAPAD markers

Detecting of high levels of polymorphism is One of the most important features of the RAPD technique, and this feature has been met in the present study (fig. 2).

Five primers were screened with the DNA of the four insect species genotype. All the primers tested was generated reproducible and easily storable. RAPD profiles with some amplified DNA fragments ranging from 9 to 13 (table 1). The total number of fragments produced by five primers was 56 with an average of 11.2 fragments / primers. While the number of polymorphic ranged from 7 to 12 with an average reached 9.8 fragments 8 primers with the polymorphic percentage reached 87.04%. Although, the number of monomorphic ranged from 1 to 2 and was total of the monomorphic 7 with an average reached 1.4 fragments 8 primers with the monomorphic

percentage was 12.35%. A maximum numbers of amplicons was amplified with primer U-17 reached 13 while the minimum number of fragments was amplified with primers OPE K-02 reached 9. The highest number of polymorphic bands reached 12 was obtained with primer U-17; while the highest number of monomorphic bands reached 2 was obtained with primer OPE K-02 and M-32.

### Genetic distance among insect species

Data of RAPD markers scanned from 4 insect species with reproducible primers were used to genetic distance and similarity value co-efficient. A maximum genetic reserve value was observed between (1) and (4), (2) and (4) reached 0.547 with less similarity value reached 45%. While a lowest genetic distance value was observed between (2) and (3) reached 0.435 with high similarity value reached 56% (table 2).

### Genetic relationships as revealed by RAPD markers used dendrogram

To determine the genetic relationships among four insect species genotype, the scoring data were used to calculate the similarity matrices. These genetic likeness matrices were then used in the cluster analysis to generate a dendrogram using in the cluster analysis UPGMA analysis. The cluster tree analysis (fig. 3) showed that the insect species were broadly divided into two main

groups A and B with genetic similarity reached 30%. A group including (1) *T. granarum*. B group was divided into two sub-cluster B1 and B2 with genetic similarity reached 36%. The first sub-cluster (B1) including only *C. maculatus* (4) species and the second sub-cluster (B2) included two species *S. Oryzae* (2) and *T. castaneum* (3) with high genetic similarity reached 48%.

## Discussion

RAPD technique took an important place in insect researchers, such as Ecological research that provides invaluable information on population structure, speciation, gene flow and genetic diversity and explanation on insect diversity based on their interaction with environmental factors, either biotic or a biotic, many times, molecular marker data help to distinguish between different species while no other comprehensive way available to do so. In addition, one of the most appealing applications of molecular markers in insect studies is the insect-plant interaction. Using RAPD-PCR with pooled DNA from different strains of Asian rice gall midge, distinct loci specific to individual strains were identified (Behura *et al.*, 2001). Insecticidal resistance is another focus in entomological research that has agricultural importance, molecular markers are used insecticides by using DNA markers, and genetic loci have been mapped in lesser grain borer, *Rhyzopertha dominica* Fabricius. that determines high-level resistance to phosphine (Schlipalius *et al.*, 2002). Also, RAPD markers have been used to generate genetic maps for many insects like beetles (Beeman and Brown, 1999; Riudavets *et al.*, 2010). Genetic Diversity among some insect species, In Florida six population of *Diaprepes abbreviatus* L. was obtained using protein and random amplified polymorphic DNA – polymerase chain reaction (RAPD – PCR ) markers, the data showed significant differences among populations (Bas *et al.*, 2000).

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