Studying Genetic Polymorphism and effect of Geographic Site in Dubas bug (*Ommatissus lybicus*) by using RAPD Technique

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Abstract

The present study aimed to investigate the genetic diversity in individuals of Ommatissus lybicus bug and the effect of their geographic site. Samples have been collected from Babel and Karbala governorates (10 indv. for each site), using Random Amplified Polymorphic DNA (RAPD) markers, which were applied to the total DNA isolated from these individuals by using ten primers; all primers showed polymorphic band patterns in both groups of the insect. Primers varied in revealing the polymorphism, the highest efficiency in bands producing was 22% with primer OPM14 in Husainia (Karbala) samples while the highest discriminating power in polymorphic bands producing was 16.4% with primers OPG02, OPF09 in Hilla samples (Babel). The lowest percentage efficiency and discriminating power in polymorphic bands were registered in primers OPF09 and OPM14.

Cluster analysis based on RAPD bands patterns was performed using Jaccard scale for genetic similarity, for each location samples and for them together. All the samples isolated in tow major group indicate that Hilla samples are more related to each other than Husainia samples as they have seven individuals in minor group with lowest genetic distance in between which was 10-28%(72-90% similarity), while Husainia samples have eight individuals with lowest genetic distance between 20-64%(80-36 %similarity). The conclusion of this study refers that the individuals of both samples are related to the rest of the individuals in same group rather than to the other group.

Keywords: Dubas bug *Ommatissus lybicus*, RAPD, Biodiversity.

Introduction

Date Palm tree (*Phoenix dactylifera* L.) represents life in desert environment as it can tolerate heat, drought and salinity more than many other fruit trees[1]. One of three palm trees in the world are in Arabic region, responsible for 67% of global product of dates[2,3]. In Iraq and Arabic countries, date tree is infected by many diseases and pests alongside with economical effects[4]. The most important pest is Dubas Bug (*Ommatissus lybicus*) which in severe infection condition causes high losses in yield quantity and its quality up to 50%[5]. Adults and nymphs suck plant syrup from all plant parts even the fruit and thus making the parts pale and yellow, other indirect damage is secreting of honeydew, the accumulation of honeydew causes pores plugged, reduces respiration, photosynthesis and loss of water, aggregation of dust and growth of mold and fungus[6]. The first record of Dubas bug in Iraq was in Bassrah in 1919[7]. It does not have an economic effect until 1935 or 1936[8].

Howard[9] mentions that the spread of insect could be caused by transferring or moving small palm trees between different regions, Zink[10] refers a wide range of parameters such as geographical, geological, environmental and genetic aspects which affect the structure and distribution of insect populations. Studies on Dubas bug discussed many sides of insects like the study of efficiency of mineral oil and ca'aoline as a replacement of Diazinon in control[11] and estimating the relationship between the amount of honeydew with date palm variety[12]. The aim of this study is to detect genetic diversity and effect of geographic site in Dubas bug collected from two governorate of Iraq (Babel and Karbalaa), considered as hot spot of infection by using PCR\ random amplified of polymorphic DNA **(**RAPD) technique.

Material and Methods

Insect samples: 10 sample of insect individuals were collected from Babel governorate / Hilla and Karbala governorate/ Husainia.

Genomic DNA isolation from Dubas bug: Genomic DNA was isolated by using salting out method described by Aljanabi and Martinez[13].

Preparation of RAPD markers Reactions: RAPD reactions were performed in an Eppendorf master cycler PCR machine as described by Williams et al [14]. According to the recommendations of Bioneer company, the supplier of PCR PreMix of total volume 20 µL for each reaction contains *TOP* DNA polymerase 1U, dNTPs 250µM/ each, Tris-HCl 10 mM, KCl 30mM, $MgCl_2$ 1.5 mM and stabilizer and tracking dye. Prepared Template DNA 50ng/ µL and primer 10 pmol were added. The PCR machine determined according to following program: an initial denaturation 1 minute at 94C˚, followed by 36 cycles of 1 minute at 92C˚, 1 minute at 36C˚ and 2 minutes at 72C˚ with final extension of 72C˚ for 10 minutes. PCR produces electrophoresed Agarose gel 1.2%. Table 1 shows the primers and their sequences from 5' to 3' end (Operon Technologies company) which have been used in this study.

Table 1
Primers used in this study and their sequences from 5' to 3' end.

Primer	Sequence
OPA04	AATCGGGCTG
OPB14	TCCGCTCTGG
OPF02	CAGGATCCCT
OPF09	CCAAGCTTCC
OPG02	GGCACTGAGG
OPG08	TCACGTCCAC
OPH02	TCGGACGTGA
OPM14	AGGGTCGTTC
OPN16	AAGCGACCTG
OPS17	TGGGGACCAC

Agarose Gel Electrophoresis: Electrophoresis was performed as described by Maniatis et al[15].

RAPD Products Analysis: RAPD products were calculated and analyzed. The present band takes symbol (1) and the absent band takes (0), Cluster analysis based on the results of RAPD technique was performed using Jaccard scale for genetic similarity between studied samples by Un weighted pair-group method of PAST ver. 1.91.[16]

Primer efficiency (E.) was calculated by equation:

P.E.= No. primer bands / No. total bands x 100

and discriminating power (D.P.) by equation:

D.P. = No. primer polymorphic bands / No. total polymorphic bands x 100

according to Grudman et al[17].

Results and Dissection

Random Amplified Polymorphic DNA (RAPD) markers Reactions: All the 10 RAPD primers used in this study gave polymorphic band patterns in both group of insects, but they varied in their efficiency to reveal polymorphisms observed by calculated efficiency in the band producing (E.) and discriminating power (D. P.) to produce polymorphism bands. The highest efficiency was 22% with primer OPM14 in Husainia samples, while the highest D.P. was 16.4% with primers OPG02, OPF09 in Hilla samples. The lowest E. and D.P. were registered in Hilla samples 3.4% and 5.9% in primers OPF09 and OPM14 respectively. Results of E. and D.P. for all primers and numbers of total and polymorphic bands are shown in tables 2 and 3 for each group of insects respectively.

Table 2
Total and polymorphic bands and Efficiency and Discriminating power\ Hilla samples.

Primer	Total bands (aver.)	Polymorphic bands	Efficiency %	Discriminating Power %
OPA**04**	3-1	6	5.7	8.9
OPB14	3-4	5	7.6	7.4
OPF09	2-7	11	13.4	16.4
OPG02	2-9	11	17.3	16.4
OPG08	1-5	7	9.6	10.4
OPH02	1-5	8	9.6	11.9
OPM14	3-6	4	11.5	5.9
OPN16	1-5	6	9.6	8.9
OPS17	1-8	9	15.3	13.4

Table 3
Total and polymorphic bands and Efficiency and Discriminating power\ Husainia samples

Primer	Total bands (aver.)	Polymorphic bands	Efficiency %	Discriminating Power %
OPA04	1-4	4	14	16
OPB14	2-3	3	11.1	12
OPF09	2-3	3	11.1	12
OPG02	1-3	3	11.1	12
OPG08	1-2	2	7.4	8
OPH02	2	2	7.4	8
OPM14	2-6	4	22.2	16
OPN16	1-4	4	14	16
OPS17	1-4	4	14	16

Each complementary sequence of a primer in genomic DNA produced a band on Agarose gel, so the number of the total bands equals to the complementary sites of the primer and any absent band on gel with any isolate, variety, line or individual compared with group meaning lake of annealing site for primer on genomic DNA and this is the base of polymorphisms in individual, species, varieties and population[18,19]. EL- Fiki[19] mentioned that there is any slight change in primer sequence caused by large polymorphisms in RAPD patterns. According to Botestien et al[20] and Smith and Smith[21], the polymorphism is in different situations like absence or appearance of a band (marker) in one sample only compared to other samples and the most type of polymorphism is sharing the appearance of band in many samples and absence of same band in other samples.

From this point, the samples that have unique single band were considered as special marker. From analyzing the results of this study, we can notice such bands are less than other studies: 4 bands in Husainia samples and 11 in Hilla samples. In the study of detection of polymorphism in different isolates of Rhizobia, one isolate had 6 unique bands[22]. So, we can conclude the polymorphism in between this samples (individuals) is low which corresponded with results of Khidr et al[23] in the study of two groups of *Goniozus* wasps from two different regions, they were more closely related to each other than to the other group.

In general, the polymorphism between the two groups of Dubas Bug in this study revealed that the special genetic patterns to each group in spite of the number of polymorphic bands varied between the two groups which were 67 polymorphic band Hilla samples, while it was only 25 band in Husainia samples and that will affect the discriminating power of polymorphic band to each primer[17].

Dendogram for Dubas Bug samples: RAPD results were invested to draw genetic relationship tree (dendogram) according to Jaccard coefficient for genetic similarity, for Hilla samples and Husainia samples separately and for both samples as presented in figure 1, 2 and 3. From figure 1 and 2, each group of samples has been isolated into two major group which have minor groups, we can conclude that Hilla samples in spite of their high polymorphism, were more related to each other. As 7 of them (5, 6, 7, 1, 8, 2 and 3) were isolated in minor group with less genetic distance between 10 -28 %, which means highest genetic similarity (90- 72 %). Husainia samples have 8 of them in minor group with genetic distance (20-64 %) meaning a genetic similarity of 80- 36%.

The genetic similarity is calculated according to Nei and Li[24] equation which is dependent on the presence of common bands between each tow couple of samples, meaning in situation of two identical DNA, the genetic distance will be equal to zero and genetic similarity is 1(100%), that means the presence of high genetic similarity between the samples referring to that they share the same alleles and that how genetic relationship is established (25).

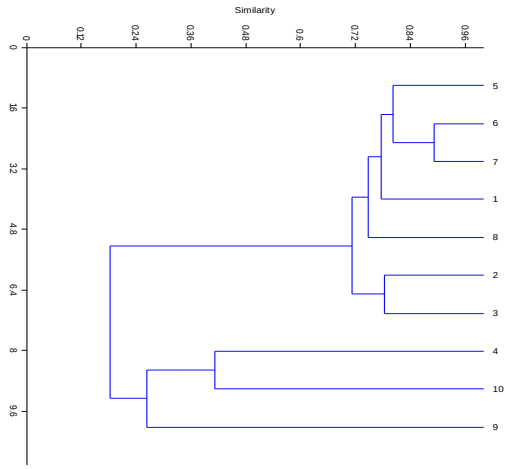

Figure 1: Dendogram showing relationships between Hilla samples.

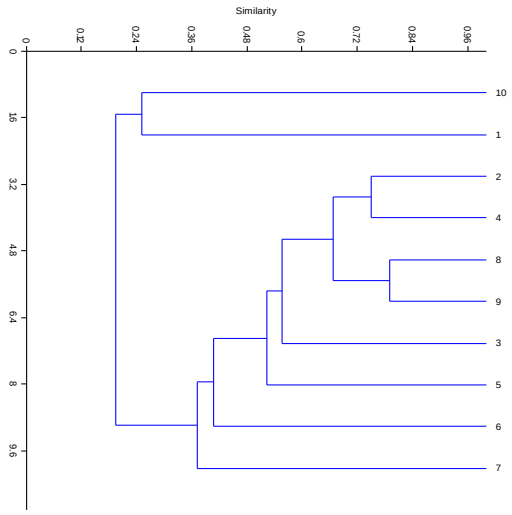

Figure 2: Dendogram showing relationships between Husainia samples.

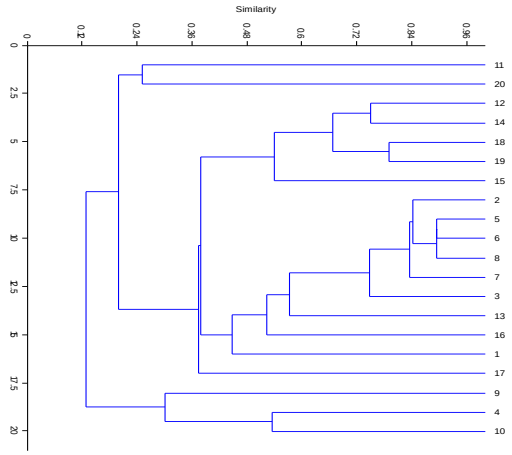

Figure 3: Dendogram showing relationships between Hilla (1-10) and Husainia (11-20) samples

Figure 3 represents that each group of insects keeps their profile of polymorphism and leads to conclusion that the two groups of dubas bug are more relative to each other within the same group more than to other group, which have different condition for each region as environmental elements, geographical and geological nature, agricultural treatment, in addition to their genetic origin[10,26]. Dubas bug is a lazy insect which prefers young frond and shadow places as palm tree, spends all its life cycle in frond[27,28].

Carvalho[29] refers to that a successful study from genetic, ecological and evolutionary sides, can be achieved by using suitable and available molecular marker, which is considered important indicator for the relationship between individual and populations. So, the importance and ability of DNA marker and RAPD technique to detect genetic distance between the studied samples represent and appear as complimentary sequence of primer with template genomic DNA, for different varieties, then to detect similar bands between tow genomes. Similar bands are high meaning less genetic distance, while less similar bands mean high genetic similarity[30].

References

1. Zohary D. and Hopf A., Domestication of plants in the old world, 3rd ed., Oxford university Press, Oxford, UK **(2000)**

2. FAO, Plant production and protection: Paper no. 35, Date production and protection, FAO Rome, Italy, 294 **(1982)**

3. El-Juhany L., Degradation of date palm trees and date and Production in Arab countries: causes and potential rehabilitation, *Aust. Jour. of Basic and App. Sci*., **4(48)**, 3998-4010 **(2010)**

4. Hussain A.E., El-Arnaouty S.A., Ghanem Kh. M. and Al-Anany M.S., Population fluctuation, biological features of Dubas Bug (*Ommatissus lybicus* DeBerg (Homoptera : Tropiduchidae) attacking date Palm trees (Phoenix dactylifera L.) and its biological control by *Chrysoperla carnea* (Steph.) larvae, *J. Plant Prot. and Path.*, Mansoura Univ., **3(6)**, 503 – 515 **(2012)**

5. Mokhtar A.M. and Nabhani S.S., Temperature-dependent development of dubas bug, *Ommatissus lybicus* (Homoptera: Tropiduchidae), an endemic pest of date palm, *Phoenix dactylifera*., *Eur. J. Entomol.*, **107(4)**, 681-685 **(2010)**

6. Klein M. and Venezian A., The dubas date Tropiduchidae, Ommatissus binotatus lybicus, threat to date palms in Israel, *J. Phytoparasitica*, **13**, 95-101 **(1985)**

7. Ramchandra R., Mem. Dep. Agric. Mesopotamia, No. 6, (Cited), 21 **(1922)**

8. Annual Report for Dubas bug on date palm and Sunn pest on wheat, control campaign, Republic of Iraq, Ministry of Agriculture, Plant Protection Directorate **(2014)**

9. Howard F., Insect pest of palms and their control, Pesticides outlook, 240-243 **(2001)**

10. Zink R.M., Methods in comparative phylogeography and their application to studying evolution in North American Aridlands, *Integrative and Comparative Biology*, **42**, 953-959 **(2002)**

11. Mahmoudi M., Sahragard A., Pezhman H. and Ghadamyari M., Efficacy of biorational insecticide against Dubas bug, *Ommatissus lybicus* (Hem.: Tropiduchidae) in a date palm orchard and evaluation of caolin and mineral oil in the laboratory, *J. of Entomol*., Society of Iran, **33(4)**, 1-10 **(2014)**

12. Shah A., Zia A., Rafi M., Mehmood S., Aslam S. and Chaudhry M., Quantification of honeydew production caused by Dubasbug on three date palm cultivars, *J of Entomol. and Zoology Studies*, **4(4)**, 47 **(2016)**

13. Aljanabi S.M. and Martinez I., Universal and rapid salt-extraction of high-quality genomic DNA for PCR-based techniques, *Nucleic Acids Research*, **25(22)**, 4692-4693 **(1997)**

14. Williams J.G., Kubelik A., Livak J., Rafalsky J. and Tingey S., DNA polymorphisms amplified by arbitrary primers are useful as genetic markers, *Nuc. Acid Rese*., **18**, 6531-35 **(1990)**

15. Maniatis T., Fritsch E. and Sambrook J., Molecular cloning: A laboratory manual, Cold Spring Harbor Laboratory, New York **(1982)**

16. Hammer Ø., Harper A.T. and Ryan D., PAST: Paleontological statistics software package for education and data analysis, *Palaeontologia Electronica*, **4(1)**, 9 **(2001)**

17. Grudman H., Schneider C., Hartung D., Daschner F. and Pith T., Discriminatory power of three DNA typing techniques for *P. aeruginosa*, *J. Clin. Microbiol*., **3**, 528-532 **(1995)**

18. Mullis K. and Faloona F., Specific synthesis of DNA in vitro via a polymerase catalyzed chain reaction, *Methods Enzymol*., **155**, 335-355 **(1987)**

19. EL-Fiki A., Genetic diversity in rhizobia determined by Random Amplified Polymorphic DNA analysis, *J. Agri. Soc. Sci*., **2(1)**, 1-4 **(2006)**

20. Botestein D., White R., Skolnick M. and Davis R., Construction of a genetic linkage map in man using restriction fragment length polymorphisms, *Am. Hum. Genet*., **32**, 314-331 **(1980)**

21. Smith J. And Smith O., Fingerprinting crop varieties, In Advances in agronomy, Sparks D.L., ed., **47**, 98-107 **(1992)**

22. Mohammed H. et al, Use of random amplified of polymorphic DNA(RAPD) markers to detect genetic polymorphism in some rhizobial strains, *Iraqi J. Agric. Res.*, **22(1)**, 147-155 **(2017)**

23. Khider S., Hardy I., Zaviezo T. and Mayes S., Development of microsatellite markers and detection of genetic variation between *Goniozus* wasp populations, *J. of Insect Sci*., **4**, 1-17 **(2014)**

24. Nei M. and Li W., Mathematical model for studying genetic variation in terms of restriction Endonucleases, Proceeding of the National Academy of Science, USA, **74**, 5269-5273 **(1979)**

25. Esselman E., Crawford D., Brauner S., Stuessy T., Anderson G. and Silva M., RAPD markers diversity within and divergence among species of dendroseris (Asteraceae: Lactuceae), *Am. J. Bot*., **87**, 591-609 **(2000)**

26. Bond J. and Stockman A., An integrative method for delimiting cohesion species: finding the population- species interface in a group of California trapdoor spiders with extreme genetic divergence and geographic structuring, *Systematic Biology*, **57**, 628-66 **(2008)**

27. Ba-Sahih J.S., Date Palm Dubas insect, *Ommatissus lybicus*, University of Hadermaot for Science and Technology, Center of Date and Date Palm **(2005)**

28. Shah A., Mohsin A., Naeem M., Farooq M., Irfan-ul-Haq M. and Hafeez Z., Biology of Dubas Bug, *Ommatissus lybicus* (Homoptera: Tropiduchidae), a Pest on Date Palm during spring and summer season in Panjgur, Pakistan, *Pakistan J. Zool*., **44(6)**, 1603-1611 **(2012)**

29. Carvalho G., Advances in Molecular Ecology, IOS press **(1998)**

30. Gonzalez J. and Ferrer E., Random amplified Polymorphic DNA analysis in *Hordeum* species, *Genome*, **36**, 1029-1031 **(1993)**.