

Molecular Diagnosis of Japanese Beetle, *Popillia japonica* Larvae Infesting Soybean Crop in Iraq

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Abstract

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This study was conducted during the period 2022-2023 at the Department of Agricultural Research, Abu Ghraib Research Station near Baghdad, within the national program for the propagation and multiplication of high-quality soybean seeds, which is grown for the first time in Iraq, at the Dibbana Agricultural Company research station in the southern Baghdad area. Weekly and monthly plant and soil samples were collected to identify pests and their population density that affect the crop in Iraq. Field studies showed the occurrence of the Japanese beetle, *Popillia japonica* on soybeans in Iraq. Diagnosis was based on insect morphology with the help of international keys and molecular tests, and identification was confirmed by the Iraqi Natural History Museum. Molecular diagnosis was registered at the NCBI GenBank under the number PP558784.1. This is the first molecular diagnosis of this pest in Iraq.

Keywords: Japanese beetle, soybean, first report, molecular diagnosis.

Introduction

The Japanese beetle, *Popillia japonica* (Coleoptera: Scarabaeidae) was recorded for the first time in America in New Jersey in 1916 (Altschul *et al.*, 1990). Japanese beetles feed on a large number of plant hosts, reaching 300 hosts belonging to 79 families (Althoff & Rice, 2022). The insect has one generation per year, where the female lays around 40-60 eggs in the soil. Eggs take 30-45 days to hatch (Crutchfield & Potter, 1995; Potter & Held, 2002), then turn into first-instar larvae and then into the second larval stage, which takes 3-4 weeks. It continues to feed until the end of the fall season, then spends the third instar in the soil at a depth of 20 cm underground and continues to feed on the roots until it emerges into adults at the end of May and the beginning of June depending on the climatic factor (Potter & Held, 2002).

Potter & Held (2002) highlighted the importance of controlling eggs in that period as they were considered the source of infestation. It is known that the Coleopteran family of beetles causes economic losses by their larval stage (1, 20); Japanese beetles cause severe economic losses by both larval and adult stages, as the larvae attack the root trees, leading to yellowing, weakness, and death of the plant and adults by feeding on leaves and flower buds, which leads to the lack of yield or lack of productivity (Cranshaw, 2007; Gould, 1963; Shanovich *et al.*, 2019).

The soybean plant, *Glycine max* L. is a fodder plant that occupies the first rank in the world. The vegetative, leaf, and pod parts are used as animal fodder. In addition to their uses as food, industrial, and medicinal purposes, they are also called the Miracal crop (Khurshid *et al.*, 2017; Lee *et al.*, 2007). Soybean is considered an economic crop characterized by seeds with a protein content of 20 to 40%

(Wolf, 1970), oil content of 14 to 24% (Clemente & Cahoon, 2009), and some other compounds (Alsaloomeh, 2023; Jasim & Aziz, 2019). Because the crop is grown for the first time inside Iraq, as part of a national program to sustain and propagate the soybean crops of the Iraqi Ministry of Agriculture, this study aimed to identify the larvae that cause severe damage to the crop. Polymerase chain reaction (PCR) is a widely used method in diagnosing many insects (Al-Saad & Aletby, 2018; Faraj *et al.*, 2020; Hade, 2020), and was used in this study to identify the Japanese beetle larvae.

Materials and Methods

Morphological characterization

After collecting larvae at various stages from soybean fields, a microscope was used to perform phenotypic descriptions of their bodies. The descriptions of the larvae were recorded and compared with the approved description keys from the Natural History Museum.

DNA sample extraction

The samples for DNA extraction were collected from larvae that were previously obtained from an infested soybean field. The larvae were thoroughly cleaned to remove soil and other impurities. They were then frozen using liquid nitrogen and ground with a mortar to obtain a fine powder. Around 25 mg of the ground tissue was transferred to a 1.5 ml Eppendorf tube for DNA extraction using the G-spin DNA Kit, produced by the Korean company Biotechnology Interon, following the recommended extraction steps from the producing company. The quality and quantity of the total DNA extracted were confirmed using a Nanodrop device. The DNA extraction process took place at Wahhaj Al-Dona laboratories.

Primers design

The primer was designed to amplify 420 bp of specific primer COX gene (Al-Musafir *et al.*, 2021) (Table 1). The sequences of *Popillia japonica* mitochondrion complete genome copies NC_038115 were aligned using BLAST software (Al-Khafaji *et al.*, 2018) to select the targeted amplification area.

Table 1. The specific primers amplify a fragment of the COX gene.

Primer	Sequence 5' to -3'	Tm (°C)	Product size (bp)
Forward	GGAGCCCCAGATATAGCTTCC	57.0	420
Reverse	AAATTGGATCTCCCCCTCCTG	56.6	

PCR reaction mixture (PCR) followed by electrophoresis

The Maxime™ PCR PreMix (i-Taq) (Cat. No# 25025, iNtRON Biotechnology, Korea) was used for PCR assay with final volume 25µl. The components of the mixture were mixed for several seconds using a combi-spin device, then the tube was placed in the thermocycler, and the amplification reaction of the DNA chain was 1 cycle at 94°C for 5 min, followed by 35 cycles of 94°C for 45sec, 58°C for 45sec, 72°C for 45sec and final extension cycle at 72°C for 7 min. The products of the amplification process (PCR) were placed in the wells of the submerged 1.5% agarose gel plate at a rate of 5 microliters per well, in addition to the standard molecular ladder solution, and the electrophoretic separation was carried out at 70 volts and 65 amps for 1.5 hours.

Sequence Analysis

Sequences analysis was processed by using Chromas version 2.6 (Gould, 1963). Moreover, the assembly of sequences (forward and reverse) was performed by using CAP3 software (Frank, 2016). Assembled sequences data were submitted to the National Center of Biotechnology Information (NCBI) for registration. The data of molecular diagnostics were identified by performing multiple alignments of each sample sequence with the NCBI database by using basic local alignment search tool (BLAST) software (Altschul *et al.*, 1990). Furthermore, a comparison between our identified sequences with the NCBI database was carried out using BLAST and the best compatible sequences to determine the similarity relationship between related COI copies.

Results and Discussion

Morphological characteristics

The larvae of Japanese beetles are spherical in shape with a yellowish-brown head (Figure 1). They have strong, chewable jaws, making them voracious feeders. The larvae are creamy white, 10.5 mm long, and curved in the shape of the letter C. They have three pairs of thoracic legs at the end of the abdomen, making their internal viscera visible. Yellow hairs can be seen on the sides at the end of the abdomen, and there is a pattern of anal hairs with a v-shaped dot pattern, which is a key distinguishing characteristic from larvae of other beetle species. The anal hairs are symmetrical, evenly

distributed, and arranged in parallel, shaped like the letter V in comparison to other beetles such as *Maladera castanea*, *Oryctes nasicornis* and *Berberomeloe majalis* (Figure 2). These hairs are soft, yellow, small, and extend over the surface of the anal area, often appearing blond or light brown in color.



Figure 1. Phenotypic diagnosis of Japanese beetle larvae from several directions.

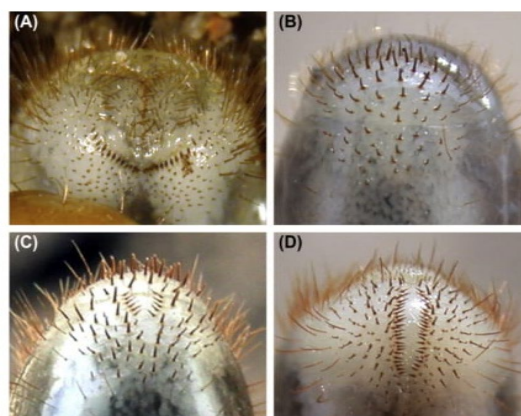


Figure 2. Comparison of the hair pattern of Japanese beetle larvae with other beetles: A = *Maladera castanea*, B = *Oryctes nasicornis*, C = *Popillia japonica*, D = *Berberomeloe majalis*.

Molecular diagnostic

The PCR test showed that the replicated samples had a 420 bp band (Figure 3). Furthermore, the PCR product was sequenced by Macrogen company, South Korea, and the sequence was registered in the NCBI GenBank under accession number PP558784.1. Moreover, samples were identified as *Popillia japonica* (Japanese beetle) (Coleoptera: Scarabaeidae). Sequences comparison showed high genetic similarity with some species belonging to the Scarabaeidae family (Figure 4). The isolate identified in this study is considered the first occurrence on soybean in Iraq. The occurrence of this pest in different regions and on other hosts, such as corn (Shanovich *et al.*, 2021), grape (Ebbenga *et al.*, 2022), or apple (Shanovich *et al.*, 2021) in Iraq needs further investigation. Because the Japanese beetle is known to cause economic losses on crops all over the world, more information on its life cycle, number of generations, and

climatic conditions suitable for its development in Iraq, as determined for other pests (Al-Jorany *et al.*, 2020; Hussien & Sadiq, 2020; Lundin, 2020), need to be determined.

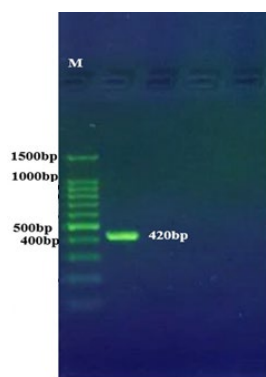


Figure 3. Agarose gel showing a PCR 420 bp product after electrophoresis. M = DNA molecular size ladder (100 bp).

The phylogenetic analysis for COI gene of the local isolate of *Popillia japonica* (PP558784.1) showed it to be identical (100%) to the Canada isolate (MH159055.1), and very closely related (99%) to other isolates from Canada (OQ622832.1), China (KJ885618.1) and USA (OP383063.1, KX687965.1). The study demonstrated the importance of mitochondrial genes in exploring the phylogenetic relationship among various clades, and deepening the understanding of the evolution of members of the family

Dermestidae (Cameron, 2014; Sizhu *et al.*, 2015; Zeng *et al.*, 2021).

It can be concluded from this study that the Japanese beetle *P. japonica* larvae were observed for the first time on soybean crop in Iraq. Measures to contain its spread are essential to reduce future potential losses due to the possible attack by this pest. Further studies are required to determine the appropriate conditions for its development in Iraq and identify the best practices to manage it.

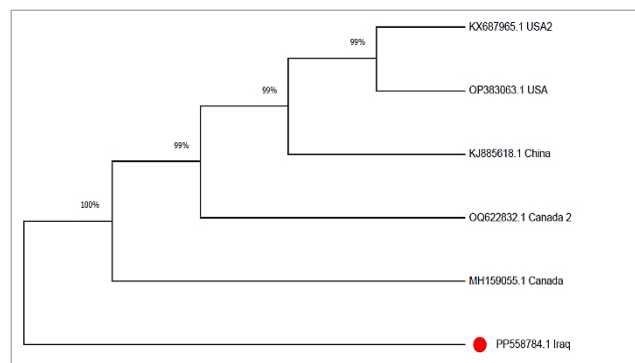


Figure 4. Neighbour-joining tree to determine the relationship among soybean field Iraqi isolate of *Popillia japonica* mitochondrial cytochrome c oxidase subunit I (COI) gene segment and similar worldwide isolates as confirmed by BLAST.

المخلص

فاضل، علاء، فريال صادق وحسن ليلو. 2026. التشخيص الجزيئي ليرقات الخنفساء اليابانية (*Popillia japonica*) في حقول محصول فول الصويا في العراق. مجلة وقاية النبات العربية، 44(1):115-118. <https://doi.org/10.22268/AJPP-001368>

أجريت هذه الدراسة خلال الموسم 2022-2023 في محطة أبحاث أبو غريب التابعة لدائرة البحوث الزراعية، قرب بغداد، وذلك ضمن البرنامج الوطني لإدامة وإكثار بذور الرتب العليا لمحصول فول الصويا والذي يزرع لأول مرة في العراق، وكذلك في محطة أبحاث شركة دبانة الزراعية في منطقة جنوب بغداد. أظهرت نتائج الدراسات الميدانية وجود الخنفساء اليابانية (*Popillia japonica*) في العراق، حيث تم تشخيصها شكلياً حسب المفاتيح العالمية، وكذلك باستخدام الاختبارات الجزيئية. تم تأكيد التشخيص من قبل متحف التاريخ الطبيعي العراقي. تم تسجيل نتائج التشخيص الجزيئي في الـ NCBI GenBank تحت الرقم PP558784.1. يعدّ هذا البحث أول تشخيص جزيئي لهذه الآفة.

كلمات مفتاحية: الخنفساء اليابانية، فول الصويا، التسجيل الأول، الكشف الجزيئي.

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