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# A Molecular study of the Cabbage White Butterfly *(Pieris rapae)* Infesting Cabbage *(Brassica oleracea var. capitata)* in Iraq

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## Abstract

A molecular study was carried out on the cabbage white butterfly *Pieris rapae*. The mitochondrial cytochrome c oxidase subunit I (COXI) gene was targeted using the Polymerase Chain Reaction (PCR) technique after extracting genomic DNA from the insect specimens. The nucleotide sequences of the COXI gene amplification products were determined. The obtained sequences were aligned and compared with previously registered sequences in the National Center for Biotechnology Information (NCBI) database. Subsequently, the Iraqi isolate was registered in the NCBI database under the name Pieris rapae isolate Tikrit-1 (Iraq) with the accession number PV012487. The results revealed a 100% sequence identity between the Iraqi and the Chinese isolates *Pieris rapae* isolate *pr1* (accession number OR491929.1), indicating a high degree of genetic similarity. The calculated genetic distance between the Iraqi isolate and other international isolates was notably low, recorded at 0.0015. A phylogenetic tree was constructed using MEGA 11 to assess the genetic relationships. The analysis demonstrated a close genetic affiliation between the Iraqi isolate and global isolates of the same species, confirming the genetic relatedness of *P. rapae* populations across different geographical regions.

***Keywords : Pieris rapae , NCBI , Genetic tree ,COXI, PCR, DNA.***

## I. Introduction

The cabbage plant *Brassica oleracea* var. *capitata* L is subjected to attacks by numerous insect and non-insect pests, which cause significant losses and a reduction in yield. Among these pests are the cabbage worm and the cabbage white butterfly, which belong to the order Lepidoptera, as well as the whitefly and leafhoppers (Alpin and Ratissima, 1975; Sharma et al., 2018). The cabbage white butterfly is considered one of the most important pests attacking cruciferous plants, causing substantial economic losses worldwide—losses that may reach up to 100%. In Asia and Europe, it causes an annual yield loss of approximately 40% (Abugila and Husien, 2023). **Infect** The cabbage white butterfly pest (*Pieris rapae*) infests all plants of the cruciferous family as well as members of the composite family such as lettuce, beet, radish, cauliflower, mustard, broccoli, and also chard, in addition to many weeds associated with these crops. It causes significant losses in these vegetables, reaching up to 38%. In India, infestation rates in cabbage fields in the Punjab region reached 33%. This pest must be controlled at the appearance of the first instar larvae, as it becomes difficult to manage once it enters its second week of development (Sarwan, 2014). The cabbage white butterfly (*Pieris rapae*) is considered a major pest of the cruciferous family, especially cabbage, where its larvae typically consume about one-third of the leaf area of a single plant. In some fields, the larvae can destroy all the leaves in cabbage fields (Konno, 2023). The cabbage white butterfly (*Pieris rapae*) is widely distributed across the globe, from tropical regions to temperate areas with cold climates. It is particularly significant in high-altitude regions with moderate rainfall. It has been recorded under various names, such as the white butterfly, in many countries around the world. The first recorded sightings were in Chile and later in South Africa (Cotoras and Pakarati, 2023). Due to the lack of comprehensive studies on the identification and characterization of this insect, attention has been directed toward its molecular identification

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using Polymerase Chain Reaction (PCR) techniques. This approach relies on mitochondrial DNA to obtain updated data on its global distribution and to study the degree of genetic similarity between its global isolates.

## II. Materials and Methods

**Insect Collection and Rearing**

In the cabbage fields of the College of Agriculture, University of Tikrit, during the 2022–2023 growing season. The larvae of the insect were collected from infested cabbage plants in several fields in the Tikrit area, located in Salah al-Din Governorate, during the month of November 2023 (Figure 1). The infestation was identified based on the feeding damage caused by the larvae on the cabbage leaves, as well as the presence of the insect larvae themselves. The infested plants, along with the larvae, were then transported to the Entomology Laboratory at the College of Agriculture – University of Tikrit.

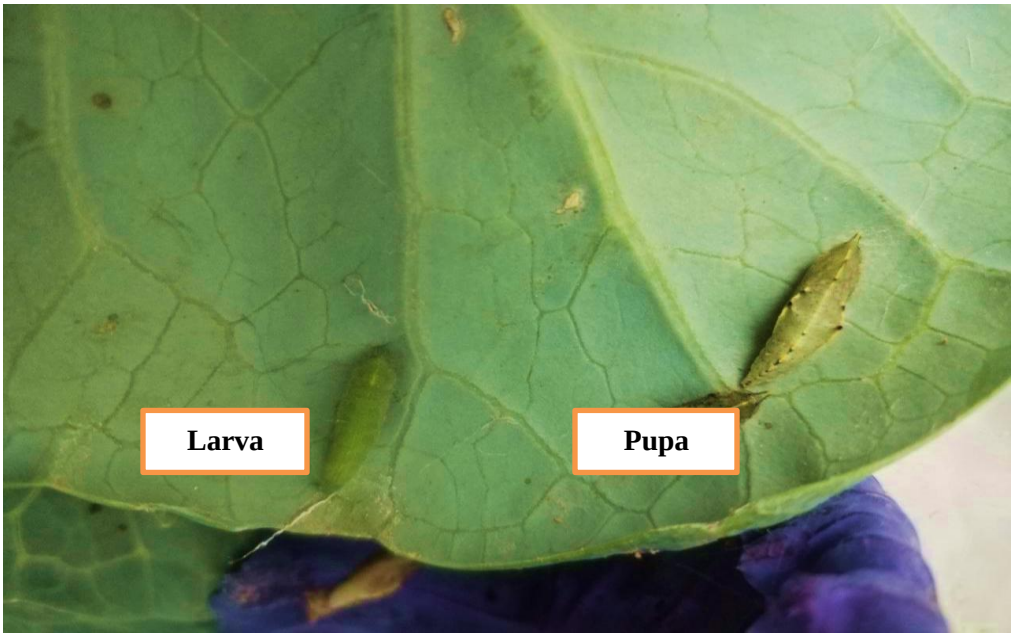


Figure (1) shows the pupa and larva of the *Pieris rapae*.

The infested fruits were subsequently placed in a glass rearing chamber with dimensions of 70×50×50 cm, specifically designed for insect maintenance. The chamber featured a single opening (7 cm in diameter) on one side, which was covered with agricultural mesh to regulate insect movement and prevent adult specimens from escaping. A 5 cm layer of washed sand was added to the bottom of the chamber to maintain the required humidity for insect development. Moisture was applied to the surface of the sand using a water spray nozzle. The chamber was carefully monitored until the emergence of adult insects, which were then transferred to the molecular studies laboratory for DNA extraction (Al-Jubouri, 2023).

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**DNA extraction**

The extraction applied using G- spin DNA extraction kit-intron biotechnology , (cat.no. 17045), and the kit contents as in the table 1below:

Table 1: the contents of G- spin DNA extraction kit that used in the DNA extraction.

| Label | Contents 50 Columns |
|---|---|
| Buffer CL | 25 ml |
| Buffer BL | 25 ml |
| Buffer WA | 40 ml |
| Buffer WB | 10 ml |
| Buffer CE | 20 ml |
| Spin Column / Collection Tube | 50 ea |
| RNase A (Lyophilized powder) | 3 mg x 1 vial |
| Proteinase K (Lyophilized powder) | 22 g x 1 vial |

**Sample Preparation:**

25 mg of powdered tissue is weighed before being inserted in a 1.5 mL microcentrifuge tube. 200 µL Buffer CL, 20 µL Proteinase K, and 5 µL RNase A were all mixed together and then incubated at 56°C for 10-30 minutes Additionally, 200 µL Buffer BL was combined and incubated at 70°C for 5 minutes. The mixture was transferred to a new tube containing approximately 350-400 µL of supernatant. 200 µL of 100% ethanol was gently mixed and transferred to a spin column. The mixture was washed with 700 µL Buffer WA, followed by 700 µL Buffer WB, and centrifuged after each wash. Following the second wash, thoroughly dried the column. 30-100 µL of Buffer CE added to the column, and incubated for 1 minute, then centrifuged to elute the DNA.

**The primers design**

The primers were designed based on the Cytochrome Oxidase 1 gene (CO1), as shown in table 2 at a final concentration of 100 pmol/µl and then 10 µl of the stock solution added in 90 µl of the free dd$H_2O$ water to reach a final volume 100 µl (Table 2).

Table 2 : The specific primer **COX** of gene

| Primer | Sequence | Product size | Ref. |
|---|---|---|---|
| **Forward** | 5'- GGTCAACAAATCATAAAGATATTG - 3' | 720 base pair | **Folmer** et al., 1994 |
| **Reverse** | 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' | | |

Method of the reaction mixture was prepared by mixing for the PCR technology for the COX1 gene. The mixture was centrifuged in a Microfuge for 4 seconds, then transferred to a thermocycler, a PCR technology device, and the following program was applied (Tables 3 and 4).

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Table3: The Components of the Maxime PCR PreMix kit (i-Taq)

| Material | Concetration |
|---|---|
| i-Taq DNA Polymerase | 5U/ µl |
| DNTPs | 2.5Mm |
| Reaction buffer (10X) | 1X |
| Gel loading buffer | 1X |

Table 4:mixing the reaction components in a PreMix kit (i-Taq) tube for PCR

| Components | Concentration |
|---|---|
| Taq PCR PreMix | 5µl |
| Forward primer | 10 picomols/µl (1 µl ) |
| Reverse primer | 10 picomols/µl (1 µl ) |
| DNA | 1.5µl |
| Distill water | 16.5 µl |
| Final volume | 25µl |

**PCR Amplification**

DNA was extracted from adult specimens of the cabbage white butterfly Pieris rapae, and its purity and quality were verified using electrophoresis in a 1.5% agarose gel. The extraction process was carried out in two stages: the first lasting 10 minutes and the second 45 minutes. Subsequently, the PCR product was electrophoresed in a 2% agarose gel. Afterwards, the nucleotide sequences of the PCR product of the COX1 gene of Pieris rapae were determined.

The DNA strand was first denaturated for five minutes at 95°C, and then it underwent thirty-five replication cycles. For each cycle, there were 45 seconds at 95°C for the double strand denaturation, 45 seconds at 58°C for the primer to bind to the template DNA, and 45 seconds at 72°C to elongate the primer. The elongation phase was then completed with a final cycle lasting 7 minutes at 72°C ( Williams , et 1990).

Using a size of 5 µl for each PCR product and DNA ladder (Cat. No. 24073), the PCR products were recognized on a 2% agarose gel (2% w/v in 1X TBE buffer) under conditions of 90 volts for 59 minutes. The bands were vitalized using red safe stain (Cat. No. 21141) and UV trans illumination.

The bands were extracted from gel and sent to Bioneer company in South Korea for sequencing.

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**Phylogenetic Analysis**

Given the importance of studying genetic trees in plant protection science, through which we can identify the degrees of genetic closeness of global isolates, and for the purpose of testing the ideal model for conducting genetic closeness, the MEGA11 program was used, as the maximum degree of similarity between the studied isolate and global isolates was adopted by coordinating the full lengths of the base sequences, which are of one length, to conduct the matching process between them and global isolates .For the sequences analyzed, including an out-group member, Mega11 was applied to ClustalW alignment and manually adjusted. A phylogenetic tree was then reconstructed using the maximum likelihood nighborhood substitution model of evolution.

## III. Results

PCR succeeded amplified 720 bp products COX1 gene for examined samples from adult the cabbage white butterfly *Pieris rapae* (Figure 2).

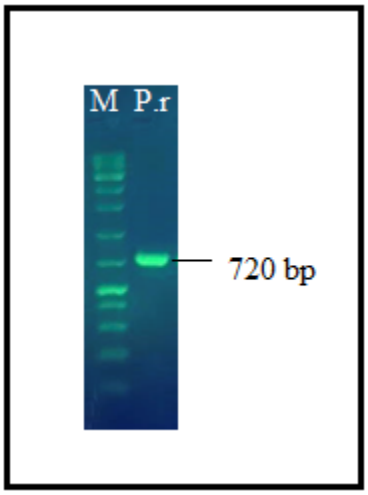


Figure 2. PCR product of COX1 gene in *P.rapae* (720 bp) visualized on 2% Agarose gel. Ladder = marker (M) (100bp) . P.r = Pieris rapae isolate Tikrit-1

The COX1 genes phylogeny tree showed that the Iraqi isolate Tikrit PV012487.1 has positioned between the Romanian isolate HQ004956.1 and a clade of six isolates from China OR491929.1, South Korea EU105213.1, Japan LC090567.1, Switzerland MVV502594.1 and two Italian isolates MVV502548.1 and MVV499237.1. The clade and Tikrit isolates' 100% match raises the possibility that the insect had similar ancestor and arrived to Iraq from East Asia by migration, shipping, or other channels (Figure 3; table 5).

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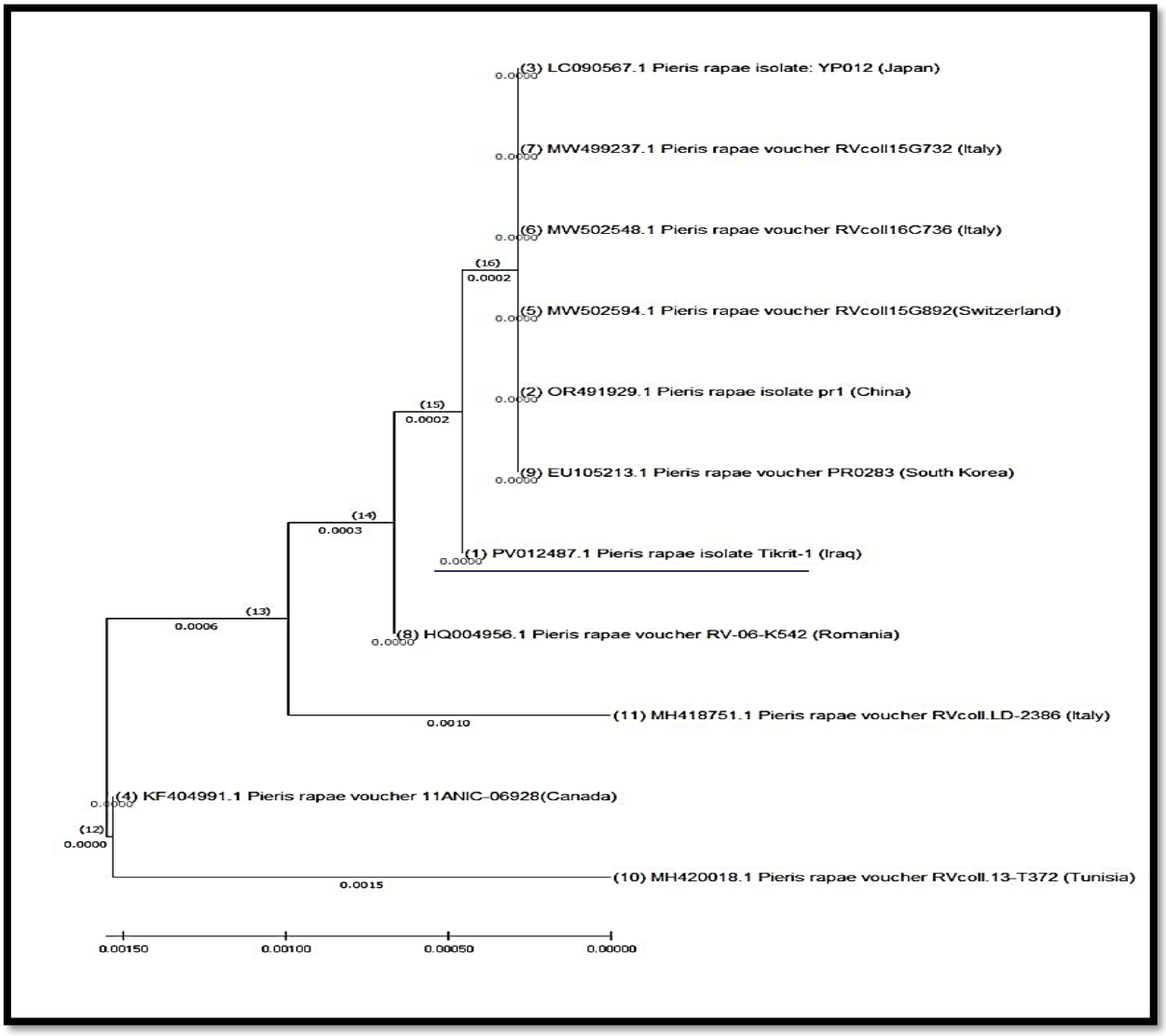


Figure 3. Maximum likelihood tree of COXI from *P. rapae* collected from Tikrit in Iraq and other Isolates from GenBank.

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Table 5 : the percentage of similarity in the cytochrome c oxidase subunit I (COX1) gene sequence of *Pieris rapae* isolate Tikrit-1 with insect Isolates recorded in the GenBank database.

| Closest Matching Insect Species & Strain | GenBank Accession Number | Country | Similarity (%) | Identified Insect in This Study | Accession Number of Identified Insect |
|---|---|---|---|---|---|
| *Pieris rapae* voucher PR0283 | EU105213.1 | South Korea | %100 | *Pieris rapae* isolate Tikrit-1(Iraq) | PV012487.1 |
| *Pieris rapae* isolate pr1 | OR491929.1 | China | 100% | *Pieris rapae* isolate Tikrit-1(Iraq) | PV012487.1 |
| *Pieris rapae* voucher Rvcoll 15G892 | MVV502594.1 | Switzerland | %100 | *Pieris rapae* isolate Tikrit-1(Iraq) | PV012487.1 |
| *Pieris rapae* voucher Rvcoll16C726 | MVV502548.1 | ltaly | %100 | *Pieris rapae* isolate Tikrit-1(Iraq) | PV012487.1 |
| *Pieris rapae* voucher Rvcoll15G7232 | MVV499237.1 | ltaly | %100 | *Pieris rapae* isolate Tikrit-1(Iraq) | PV012487.1 |
| *Pieris rapae* isolate:YP012 | LC090567.1 | Japan | %100 | *Pieris rapae* isolate Tikrit-1(Iraq) | PV012487.1 |

## IV. Discussion

The use of molecular markers in phylogenetic analysis has grown recently, and this discipline has thrived as a result of the quick advancement of data processing and DNA sequencing technologies. Genes encoded in the nucleus, including 16S rRNA, 5S rRNA, and 28S rRNA, and in the mitochondria, including cytochrome oxidase, mitochondrial 12S, cytochrome b, and organization area, have been the subject of research. Additionally, when building phylogenetic trees, exact criteria were adhered to. Molecular markers are crucial in the study of phylogeny (Patwardhan *et al*., 2014). The current study confirms earlier research on the application of procedures to evaluate mitochondrial DNA markers via the COX1 gene (Jaber *et al*., 2023). This insect has been registered in Basra province on Brassicaceae plants . Senthil and Srinivasan (2021) reported that the COX1 universal primers amplified 690 bp, but the COX1 gene specific primer amplified around 720 base pairs. Additionally, it is probably caused by haplotype. Finding genetic variation in a species can be helpful for identifying the invasion's time and location, which is in line with what Hiszczynska-Sawicka and Phillips (2014) explained. By comparing a section of the mitochondrial cytochrome c oxidase subunit I gene (COI) with other sequences from GenBank and the Barcode of Life program, genetic diversity of *Pieris brassicae* in New Zealand was discovered. The first haplotype that was produced was the same as a sequence from Spain and Romania. The second was exactly the same as a German sequence. However, none of the other reported sequences matched the third. Different clades were generated for P. rapae based on the maximum likelihood phylogenetic tree using the reference isolates P. rapae from NCBI, aside from isolate Tikrit-1 (Iraq). Its two branches, which comprise isolates from Canada and Tunisia, reflect members who are distantly related. Our study of the Pieris rapae isolate from Tikrit, Iraq, was closely related to isolates found in Japan, Italy, Switzerland, China, and South Korea. These findings demonstrated the genetic differences among the original *P. rapae* isolates. This could be the outcome of evolutionary processes occurring in those regions. Since it takes time to distinguish between intraspecific variation (isolates) and interspecific divergence (barcode gap), mitochondrial DNA is unable to

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identify newly emerging species. For this reason, we would like to draw attention to the need for additional samples in future studies utilizing nuclear regions.

Disclosure statement

The authors did not disclose any potential conflicts of interest.

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