First Morphological and Molecular Identification of the Citrus Mealybug Planococcus citri (Hemiptera: Pseudococcidae) in Diyala, Iraq Using the COX1 Gene

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Abstract

The citrus mealy bug (Planococcus citri) is a widespread agricultural pest, causing significant economic damage to horticultural crops in many regions of the world. This study aimed to conduct a preliminary morphological and molecular characterization of a local isolate of this insect in Diyala Governorate, Iraq, using DNA barcoding technique targeting the cytochrome c oxidase subunit I (COX1) gene. Samples of the mealy bug Planococcus citri were collected from orchards for DNA extraction. Genomic DNA was isolated using standard laboratory tools and techniques. Polymerase chain reaction (PCR) was performed using COX1-specific primers, and the results revealed a band of 720 base pairs, indicating successful gene amplification. Sequence analysis using the Basic Local Alignment Search Tool (BLAST) showed that the Iraqi isolate (MUM-1) shared 98.85% similarity with an isolate previously recorded from Tanzania, indicating a clear genetic affinity. Furthermore, this isolate was placed within the first evolutionary group, which included isolates from similar climatic regions. These findings confirm the reliability of the COX1 gene as an effective tool for the molecular diagnosis of P. citri. This study represents a significant scientific contribution to documenting the genetic structure of this pest in Iraq and provides baseline data essential for future phylogenetic studies and pest management strategies.

Keywords. Mealy bug, COX1 gene, PCR

Introduction

Planococcus citri commonly known as the citrus mealy bug, is a globally significant pest affecting both agricultural and horticultural sectors. It causes significant damage to a wide range of host plants, including citrus, mango, and ornamental species. The economic impact of this pest is primarily attributed to its feeding behavior, sucking sap from the bark, which weakens plants and leads to symptoms such as leaf yellowing, stunted growth, and reduced fruit quality [10]. In addition to direct damage, its honeydew secretion promotes the growth of sooty mold, further diminishing the market value of crops [13]. With a host range exceeding 60 plant species across 25 families, P. citri demonstrates a remarkable capacity to infest diverse agricultural systems, complicating control measures and posing risks to global trade. Due to quarantine restrictions in export markets [3], the pest's high reproductive rate and its ability to produce overlapping generations in warm climates exacerbate infestations, which increases the demand for effective pest management strategies [7]. Integrated pest management (IPM), which combines biological, chemical, and cultural processes to control pests, offers a sustainable approach to mitigating the impact of P. citri. Biological control agents such as Leptomastix dactylopii and parasitoids such as Cryptolaemus montrouzieri have proven effective in suppressing mealy bug populations when introduced in a timely manner [7]. Chemical

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control, although widely used, faces challenges such as insecticide resistance, requiring insecticide rotation and the adoption of new solutions, including nano-pesticides, traps and pheromones [13]. Meanwhile, molecular diagnostic tools, including DNA barcoding and real-time PCR, have revolutionized pest identification and resistance monitoring, which led to enhancing the accuracy and effectiveness of pest management strategies [4]. By combining these tools, researchers and practitioners can more effectively control crop protection and reduce the economic and environmental costs of pest management.

The life cycle of P. citri is characterized by distinct stages, including egg, nymph, pupa (in males), and adult. Females lay clusters of 300-600 eggs in waxy egg sacs, which hatch within 6-10 days under favorable environmental conditions, initiating the life cycle. Nymphs, often referred to as crawlers, are highly mobile and capable of dispersing rapidly through host plants, thus facilitating rapid infestations [1]. Male mealy bugs undergo a pupa, during which they develop wings, enabling dispersal and mating. Females remain stationary after reaching maturity, becoming immobile and focusing solely on reproduction [15]. This stationary behavior of females is one of the reasons behind the high population density of mealy bugs in infested areas. The life cycle is greatly influenced by environmental factors such as temperature and humidity. Therefore, warm, humid climates accelerate growth, leading to the possibility of overlapping generations. This characteristic facilitates rapid growth, exacerbating infestations during peak growth seasons. [16]. The behavioral adaptations of P. citri increase the complicated pest control strategies. The mealy bug's tendency to congregate in protected areas of the plant, such as leaf axils and under bark, provides a form of protection from natural predators and makes it more difficult to detect during routine pest control practices [16]. Furthermore, P. citri secretes a sticky honeydew that attracts ants. This symbiotic relationship benefits both species; the ants protect the mealy bugs from natural enemies in exchange for the honeydew, which serves as a rich food source [7 .[

Accurate identification of P. citri is essential for its effective control, especially in agricultural areas where multiple mealy bug species coexist and share similar morphological characteristics. Traditionally, morphological diagnostics have been the standard method for identifying mealy bugs. However, these methods often require specialized knowledge and detailed microscopic examination, which can be time-consuming and error-prone, especially in environments where multiple species overlap [4]. Consequently, molecular diagnostic techniques have emerged as valuable tools, providing more reliable and rapid identification capabilities. These molecular methods, particularly DNA barcoding, help overcome the challenges of morphological identification by enabling accurate and early detection of pest infestations. Furthermore, molecular diagnostics can be applied at all stages of pest development, enhancing monitoring accuracy and timing, which is critical for successful pest control [9]. Morphologically, the mealy bug P. citri exhibits several distinguishing features, most notably its oval body shape and the distinctive white waxy coating that covers its body, providing a protective shield against environmental stresses, drought, and predators [1]. This waxy secretion also helps identify the pest species, giving mealy bugs a distinctive appearance that makes them easy to recognize under various conditions. Females are typically larger, about 3 mm long, and wingless, limiting their ability to disperse compared to the smaller, winged males. Despite their short lifespan, males play a crucial role in reproduction by mating with immobile females. However, distinguishing P. citri from other mealy bug species within the Pseudococcidae family can be difficult without a detailed understanding of morphological features, such as body segmentation, wax filament arrangement, and the size and shape of the anal tubercles. Variation in these features, especially under different environmental conditions, can lead to the possibility of error occurrence in identifying and differentiating between these different species. Therefore, it requires a great deal of experience to accurately identify these pests [9]. In recent years, advances in molecular methods, particularly DNA barcoding, have revolutionized the identification of P. citri. DNA barcoding relies on conserved mitochondrial gene regions, which is highly conserved across animal species but also contains sufficient diversity to distinguish closely related species [2]. This technique has proven highly effective in can be crucial for designing more effective strategies to control this pest [17]. By identifying genetic markers associated with resistance, researchers and pest managers can track the development of resistance in mealy bug populations and adjust treatment regimens accordingly. For example, if a population is found to carry resistance genes to a particular insecticide, alternative control methods, such as biocontrol agents or different classes of insecticides, can be used to prevent further development of resistance. Furthermore, molecular diagnostics help improve management of these pests by ensuring that interventions are applied only when necessary, thereby reducing reliance on chemical treatments and mitigating their environmental impact. This integration of molecular tools into pest management strategies has made pest control programs more targeted, cost-effective, and environmentally sustainable [8]. By combining traditional morphological methods with modern molecular techniques, pest control experts are better able to identify P. citri early, monitor its spread, and apply the most appropriate control measures with greater accuracy and efficiency. Continued development and application of these diagnostic tools will be essential for managing distinguishing P. citri from other scale insects and other pests that may exhibit similar external characteristics. The use of molecular markers provides a level of accuracy and speed unmatched by traditional morphological methods, allowing the identification of P. citri at various developmental stages, including egg, nymph, and adult. The integration of molecular diagnostics into pest control programs has enhanced the accuracy and efficiency of monitoring and control measures to manage increasing populations. Molecular diagnostics also play an increasingly important role in managing insecticide resistance in P. citri. Genetic analyses, such as real-time polymerase chain reaction, enable the detection of specific resistance genes within mealybug pest populations, which

this pest in the ever-evolving agricultural landscape [11]. Therefore, the aim of the study is to conduct a preliminary morphological and molecular characterization of a local isolate of mealy bug P. citri in Diyala Governorate, Iraq, using DNA-barcoding technique for the cytochrome c oxidase subunit I (COX1) gene.

Materials and Methods

Samples of the mealy bug were collected from infested citrus orchards in Diyala Governorate, Iraq. The specimens were preserved in tubes containing 95% ethanol and transported to the laboratory for molecular analysis. Genomic DNA was extracted from adult individuals using DNA extraction Kit by G-spin technique (Intron Biotechnology, South Korea), following the manufacturer’s protocol. To confirm the success of DNA extraction, the products were subjected to electrophoresis on a 1.5% agarose gel stained with Safe-Red, and the resulting bands were visualized and compared against a standard DNA ladder. Various chemicals and molecular reagents used during DNA extraction and gene amplification steps are listed in Table 1

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Table 1. Chemicals and molecular reagents used in DNA extraction and amplification

	Material	Catalog number	Company
1	Agarose	8100.11	Conda / USA
2	Red safe staining solution	21141	Intron / Korea
3	6X Loading dye	21161	Intron / Korea
4	DNA Ladder (1000 plus bp)	24075	Intron / Korea
5	PCR Pre-Mix (i-Taq)	25025	Intron / Korea
6	TBE buffer 10X	IBS.BT004	Conda / USA
7	Primers (COX1)	-	IDT / USA
8	G-spin DNA Extraction Kit	17045	Intron Biotechnology / Korea

To amplify the COX1 gene (cytochrome c oxidase subunit I), polymerase chain reaction (PCR) was used using specialized primers, as published by [6.[

Forward primer sequence: GGTCAACAAATCATAAAGATATTG-3'-5;'

Reverse primer sequence: TAAACTTCAGGGTGACCAAAAAATCA-3'-5.'

PCR amplification was performed using Maxime™ i-Taq PreMix (Intron Biotechnology, Korea), a ready-to-use PCR master mix..

The reaction mixture contained 1.5 µL of template DNA and 1 µL of each primer. The volume was increased to 25 µL by adding sterile, nuclease-free water. Table (2) revealed the composition of the reaction mixture.

Table 2. Components of the PCR mixture used to amplify the COX1 gene.

Component	Volume (µl)	Concentration
PCR PreMix	5	ready
Primer Forward	1	10 pmol/µl
Primer Reverse	1	10 pmol/µl
DNA Template	1.5	-
Sterile distilled water	16.5	-
Total	25 µl	-

The reaction was performed using device of MultiGene OptiMax PCR (Labnet, USA), and the thermal program was applied as shown in Table (3), an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 45 seconds, then annealing at 58°C for 45 seconds, and extension at 72°C for 45 seconds. The reaction was completed with a final extension at 72°C for 7 minutes.

Table 3. Thermal program used for COX1 gene amplification

Stage	Temperature (°C)	Time	Number of cycles
Initial (Initial denaturation)	95	5 minutes	1
Denaturation	95	45 seconds	35
Annealing	58	45 seconds	35
Prolongation	72	45 seconds	35
Final prolongation	72	7 minutes	1

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After the completion of the reaction, the PCR product was transferred to a 2% agarose gel and stained with Save Red dye. The results were visualized using a gel documentation system equipped with ultraviolet (UV) illumination to determine the size of the resulting band. The laboratory procedures were carried out using a set of modern equipment, as detailed in Table (4.(

Table 4. Devices used

Number	Device	Country of origin	Company
1	PCR (MultiGene OptiMax) Device	USA	Labnet
2	Electrophoresis Device	USA	CBS Scientific
3	Gel documentation Device	USA	Labnet
4	UV Transmission Device	France	Vilber Lourmat
5	Microspin Centrifuge	Germany	Biosan
6	Dry Block Heating Device	Germany	Bio San
7	Vortex Device	Germany	Digsystem

The positive PCR results were sent to Pioneer Company in South Korea for nucleotide sequence determination. The sequences were analyzed using the BLAST tool in the National Center for Biotechnology Information (NCBI) database. The Iraqi sequence was compared with global isolates and included in a phylogenetic tree. The gene sequence was officially registered under the accession number PQ328963.1, under the name of the Iraqi isolate MUM-1.

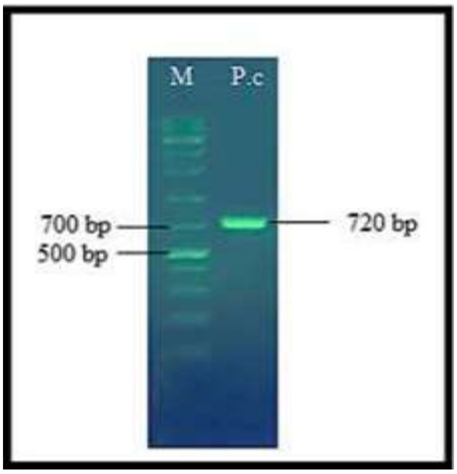

Figure 1. PCR product with a band size of 720 base pairs

Results and Discussion

The results of the study demonstrated the success of DNA extraction from P. citri, with sufficient quality to amplify the target gene. A portion of the COX1 gene was amplified using PCR, resulting in a clear band of 720 base pairs on an agarose gel, indicating the effective and precise amplification of the target sequence (Figure 1.(

The sequence of the Iraqi sample was analyzed using the BLAST tool, and the results showed a 98.85% similarity with a registered isolate from Tanzania (OP776140.1). The Iraqi isolate was also officially registered in the NCBI database under the accession number PQ328963.1. Table (5) illustrates the similarity percentage between the two isolates. This indicates a clear genetic closeness between isolates from Iraq and East Africa. These findings support the reliability of PCR using the COX1 gene as an accurate method for genetic identification and place the Iraqi isolate within Group I, which includes isolates from similar climatic regions.

Table 5. Molecular identification of the Iraqi Planococcus citri isolate (MUM-1) based on COX1 gene sequence similarity with a registered Tanzanian isolate from the NCBI database

Global accession number of the identified insect in this study	Identified insect species in this study	Similarity percentage (%)	Country	Accession number	Species and strain of the insect with the highest match
PQ328963.1	*Planococcus citri* isolate MUM-1	98.85%	Tanzania	OP776140.1	*Planococcus citri* isolate TMO2

Sequence comparison also revealed that the Chinese isolate (KP692647), reported by [18], shares a 98.7% similarity with the Iraqi isolate and is also placed within the same clade. That study used COI barcoding techniques to analyze genetic variation among P. citri isolates in China, which supports the findings of the current research and indicates that this species exhibits limited genetic diversity in certain geographical regions.

On the other hand, a study conducted by [9] from Malaysia showed positive results for amplifying the COI, 18S rDNA, and ITS2 genes of P. citri isolates using integrated molecular analysis. These isolates recorded high matching rates with the Iraqi isolate, supporting their inclusion in the first group. Although the registry number of the Malaysian isolates is not available on the NCBI website, their reliance on a similar methodology strengthens the validity of the comparison results. In contrast, isolates from other countries, such as Egypt, showed less similarity and were placed in the second group. An Egyptian study [5] indicated that Egyptian P. citri isolates amplified with the COI gene showed a different genetic pattern than Asian isolates.

These results are consistent with previous Iraqi studies, such as the study by [14] using COX1 from the first documented isolate of Cadra cautella in Iraq, which was registered with the NCBI under numbers PP916775 and PP921921, enhancing the reliability of this methodology. A study by [12] on Taenia saginata Cyst also showed genetic similarity between Iraqi isolates and their Iranian and Korean counterparts using the same gene, reflecting the effectiveness of COX1 as a phylogenetic classification tool in Iraq and the region. Therefore, the Iraqi isolate MUM-1 represents a new qualitative addition to the global sequences of P. citri and opens the way for more comprehensive comparative studies that include other Iraqi provinces. This will support the development of a comprehensive genetic database essential for strategic pest management that will contribute to the scientific and strategic management of this insect species

Conclusion

This study demonstrates that the COX1 gene is a reliable molecular marker for identifying Planococcus citri in Iraq. PCR and BLAST analyses showed high genetic similarity between the Iraqi isolate (MUM-1) and a Tanzanian reference, suggesting a possible evolutionary relationship among populations in similar climates. These results underscore the importance of integrating molecular tools with traditional morphological methods in pest identification, particularly within the morphologically similar Pseudococcidae family. Registering the Iraqi isolate in the NCBI database enriches the global genetic dataset and supports future research on this economically significant pest.

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