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Population structure and genetic diversity of *Tetranychus urticae* (Acari: Tetranychidae) in various habitats assessed by ISSR and COI analyses

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ABSTRACT

This study investigated the population structure and genetic diversity of the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), across three different environments: farm, greenhouse and laboratory. Analysis of a 632-nucleotide fragment of the mitochondrial COI gene revealed four distinct haplotypes, with one haplotype shared among all populations, suggesting a common genetic origin. The farm population exhibited the highest haplotype diversity (Hd = 0.699), while the greenhouse population showed minimal diversity and the laboratory population exhibited complete uniformity. Genetic distance analysis confirmed these differences, showing significant divergence between the farm population and the more genetically uniform greenhouse and laboratory groups. ISSR marker analysis supported these findings, with the farm population having the highest number of alleles (4) and effective alleles, as well as elevated values for Shannon's information index and expected heterozygosity. Principal Coordinates Analysis (PCoA) revealed clear genetic clustering: laboratory samples formed a tight isolated cluster, greenhouse samples showed moderate clustering and farm samples were broadly dispersed. These patterns indicate that rearing environments significantly influence the genetic structure of *T. urticae*, with open field conditions promoting genetic diversity, while controlled environments lead to reduced variation. These findings are important for understanding pest adaptability and optimizing integrated pest management (IPM) strategies.

KEYWORDS

Allelic variation, haplotype, population genetics, Principal Coordinates Analysis, spider mite

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INTRODUCTION

The genome of the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae), one of the significant agricultural pests with widespread global impact on crop production, is an extremely significant subject for scientific research (Goka and Takafuji 1995; Hoque *et al.* 2010; Grbić *et al.* 2011; Belay *et al.* 2018; Osman *et al.* 2019; Assouguem *et al.* 2022). This pest is common in greenhouses and agricultural crops, with origins in Eurasia (Grbić *et al.* 2011). It has a very wide host range and a tiny body. Its rapid population growth can cause serious damage, including up to 50% yield loss in crops like tomatoes (Navajas 1998; Jayasinghe and Mallik 2011; Li *et al.* 2015; Bensoussan *et al.* 2016; Jakubowska *et al.* 2022; Lee-Rodriguez *et al.* 2024).

Accurate species-level identification of harmful and beneficial insects is at the heart of crop protection schemes. Historically, researchers dealing with Tetranychidae and Phytoseiidae mites have employed morphological characteristics for identification purposes (Ben-David *et al.* 2007). Despite that, the microscopic nature of such mites as well as poor morphological characteristics that can be analyzed have posed challenges with their identifications. Consequently, over the last two decades, there has been more use of DNA-based identification methods, using DNA sequence similarity analysis and phylogenetic relationships in species identification (Navajas and Fenton 2000; Hurtado *et al.* 2008; Pérez-Sayas *et al.* 2015; Gómez-Martínez *et al.* 2020). Although molecular approaches such as COI barcoding were initially employed as reliable tools to resolve taxonomic ambiguities within the family Tetranychidae, their application has gradually expanded to investigate intraspecific variation and population-level genetic patterns. In the present study, this molecular marker was specifically used to analyze population genetic structure, with the primary aim of uncovering patterns of genetic variation rather than merely defining taxonomic boundaries.

Genetic diversity and population dynamics are important in the effective design of pest management programs, especially in integrated pest management (IPM) and biological control programs. Therefore, many studies based on mitochondrial cytochrome *c* oxidase subunit I (mtCOI) gene sequencing have examined the genetic diversity of populations of different species from various geographical regions (Navajas and Boursot 2003; Ros and Breeuwer 2007; Abdolahadi *et al.* 2022). Research has shown that genetic diversity plays a crucial role in the local adaptation of populations to environmental stresses, including host plant preferences and the development of resistance mechanisms to pesticides (Matsuda *et al.* 2013, 2014; Hada *et al.* 2016.) Molecular markers such as RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), RFLP (Restriction Fragment Length Polymorphism), SSR (Simple Sequence Repeat), ISSR (Inter-Simple Sequence Repeat), and DALP (Direct Amplification of Length Polymorphism) have been widely used for assessing genetic diversity and species differentiation in mites. Two molecular markers, mtCOI and ITS2 (Internal Transcribed Spacer 2), have become pillar tools in contemporary taxonomy, transforming our capacity to define tetranychid mites with unparalleled accuracy. Combining these methods provides a comprehensive approach for phylogenetic studies and the development of biological control strategies against mites (Osakabe and Sakagami 1994; Edwards *et al.* 1998; Cruickshank 2002; Navajas *et al.* 2002; Zhang *et al.* 2008; Zou *et al.* 2011).

These genetic markers have been invaluable in delineating among numerous species in this difficult family of arachnids, allowing investigators to determine clear-cut taxonomic limits where conventional morphological techniques are inadequate (Navajas *et al.* 1992; Xie *et al.* 2006). The use of the mtCOI gene in the identification of agriculturally significant mite species is hampered by two principal obstacles: (1) insufficient COI sequence data in public databases, and (2) skepticism regarding the validity and authenticity of available sequences (Ros and Breeuwer 2007; de Mendonça *et al.* 2011; Tixier *et al.* 2012; Pérez-Sayas *et al.* 2022). Until September 2021, GenBank contained over one million Acari sequences; however, less than 10% are agriculturally significant mites. This study not only provides a better understanding of the population structure and patterns of genetic variation in the two-spotted spider mite, *T. urticae* (Acari: Tetranychidae), but also highlights the role of different environmental conditions as determinants shaping population genetic structure (Tixier *et al.* 2012).

MATERIAL AND METHODS

Sample collection

Three distinct populations of the two-spotted spider mite (*T. urticae*) were collected from different regions of Iran. The sampling sites represented diverse environmental conditions and included: the Zoology Department of the Iranian Research Institute of Plant Protection, a strawberry greenhouse in Kurdistan Province, and an open-field bean crop in Mazandaran Province. Permanent slide mounts were prepared using Hoyer's medium and examined under a light microscope, following the protocol described by Zhang (2003). Immediately after collection, all samples were preserved in 95% ethanol and stored at −20 °C to ensure the integrity of DNA for subsequent genetic analyses.

DNA extraction protocol

DNA extraction was performed following a modified CTAB-based (cetyltrimethylammonium bromide) method (Hosseininia *et al.* 2019) adapted from Doyle (1991) and Ros and Breeuwer 2007. The integrity and cleanliness of the DNA were assessed using 1.5% agarose gel electrophoresis and a UV spectrophotometer. A segment of the COI gene was amplified via PCR with the primers 5'-TGATTTTTTGGTCACCCAGAAG-3' and 5'-TACAGCTCCTATAGATAAAAC-3 (Espitia *et al.* 2024).

PCR amplification [mtDNA (COI)]

The polymerase chain reaction (PCR) was conducted at a total volume of 25 µL. Each reaction mixture contained 2 µL of template DNA, 2.5 µL of 10X PCR buffer, 1 µL of $MgCl_2$ (50 mM), 0.5 µL of dNTPs (10 mM), 0.3 µL of Taq DNA polymerase (5 U/µL), and 1 µL of each primer (10 µM). The thermal cycling conditions included an initial denaturation step at 95 °C for 3 minutes, followed by 40 cycles of denaturation at 95 °C for 30 seconds, annealing at 48 °C for 30 seconds, and extension at 72 °C for 60 seconds. A final extension step was carried out at 72 °C for 7 minutes to complete the amplification process İnak (2021).

Data Analysis

Sequence alignment and genetic analyses were conducted using MEGA version 7.0.26, employing the ClustalW algorithm (Kumar *et al.* 2016). The Kimura 2-parameter model Kimura (1980) and Maximum Likelihood method with 1000 bootstrap replicates were used for phylogenetic analysis. Nucleotide composition and genetic distances were calculated within MEGA. Population genetic parameters, including nucleotide diversity, haplotype identity, and neutrality tests, were assessed using DnaSP version 5 (Librado and Rozas 2009). Haplotype relationships were visualized through network analysis in PopART version 1.7.2 (Leigh and Bryant 2015).

PCR amplification (ISSR)

Three microsatellite markers originally developed by Navajas *et al.* (1998, 2002) were used to assess genetic diversity in three populations of *T. urticae* (Table 1). ISSR primers were employed for DNA amplification in PCR reactions. Each PCR was performed at a final volume of 25 µL, containing 2.0 mM $MgCl_2$, 0.25 mM of each dNTP, 0.3 µM of each primer, 0.75 units of Taq DNA polymerase, and 20 ng of template DNA. The annealing temperature for each primer was optimized individually to improve amplification efficiency. The PCR protocol consisted of an initial denaturation at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 45 seconds, annealing at the optimized temperature for 45 seconds, and extension at 72 °C for 90 seconds. A final extension was performed at 72 °C for 5 minutes to complete the reaction. Each PCR was repeated twice to ensure the reproducibility of the results, and only stable and reproducible bands were considered for further analysis. Amplified products were separated on a 1% agarose gel at 100 V, and gel images were recorded for evaluation.

Table 1. Primer sequences and locus characteristics originally developed by Navajas *et al.* (2002).

ISSR locus	Repeat Motif	Primer Sequences (5'–3')	Allele Size (bp)	Annealing Temperature (°C)	Number of Alleles
Tu1	$(GT)_{30}$	F:GATGTAAAGGAGCGCAAAGG R: CATTGTTTGTCGATTTCCTCTC	152	57	4
Tu11	$(GT)_{29}$	F:CGTGTACAATCAGTCAACATCC R: TGGACTTTTTAACGTGGCT	154	57	5
Tu35b	$(TGA)_8$	F:AATGGAATGAGTTATCGTTGGG R: CTTCCCGAAGGCTGTTGATA	108	53	3

Data Analysis

The banding patterns of ISSR markers were scored based on the presence or absence of a band in samples. Genetic diversity indices (N_a, N_{ae}, G, H_e, H_o, FIS) were calculated using GenAlEx 6.5. (Peakall and Smouse 2006). To assess the relationship between genetic and geographic distances, a Mantel test with 999 permutations was performed. Nei's genetic distance was used to construct a pairwise distance matrix, and PCoA was applied to visualize genetic structure. All analyses were done in GenAlEx, and graphs were prepared using Excel and R version 4.2.0 R Core Team (2024).

RESULTS

Mitochondrial COI gene sequences from *T. urticae* were extracted and analyzed. These sequences were compared to reference sequences in the NCBI database, showing a high similarity of 95% to 100%, which confirms the conserved nature of this genetic marker (Fig. 1). The newly obtained sequences have been submitted to GenBank under accession numbers PV545057 and PV566912–PV566919. This molecular dataset serves as a valuable resource for precise species identification, population genetics studies, and the development of integrated pest management strategies.

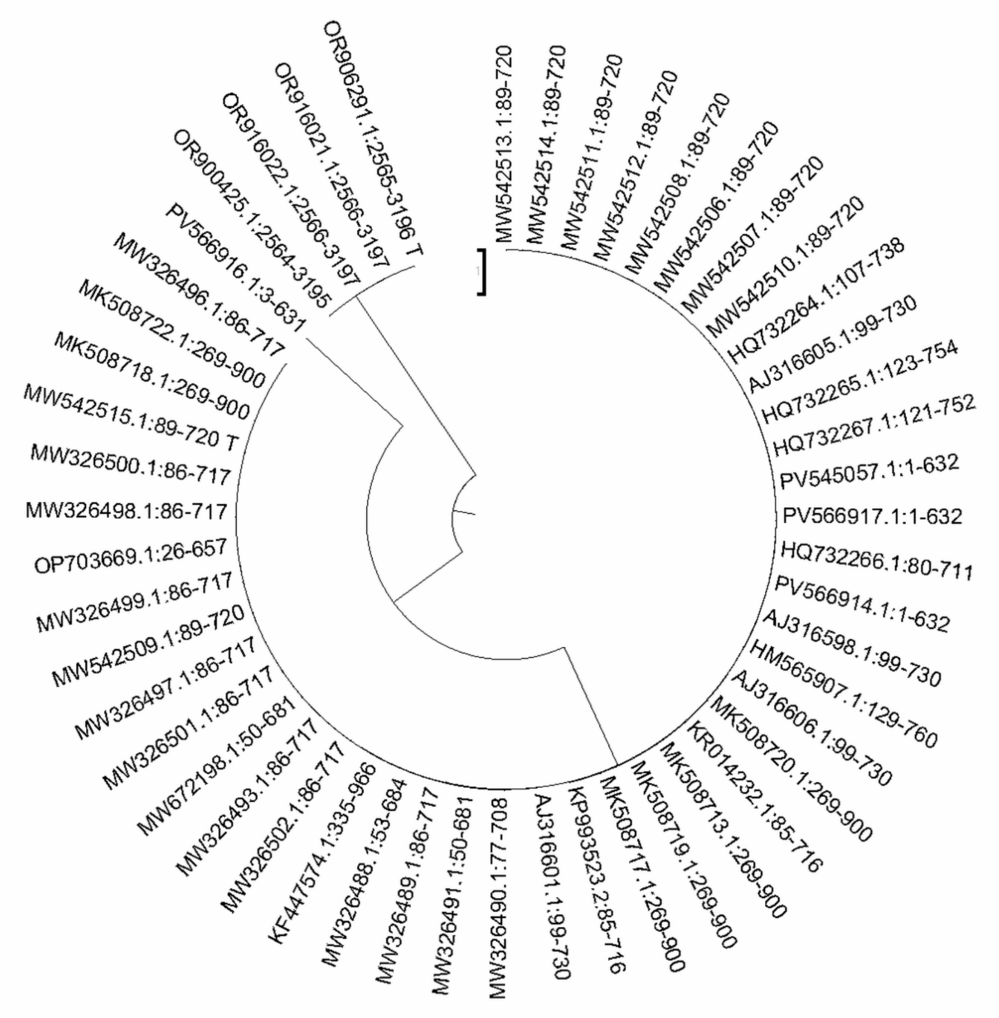

Figure 1. COI gene sequence similarity of *T. urticae* based on NCBI BLAST analysis.

Nucleotide composition

The comparative analysis of nucleotide sequences in *T. urticae* under different growth conditions indicates that the nucleotide composition remains relatively stable across all environments (Fig. 2). Cytosine (C) was consistently observed at a fixed percentage of 12.8% with no variation. Thymine (T) showed slight variation, with the highest proportion in farm samples (43.0%) and the lowest in greenhouse samples (41.9%). Adenine (A) varied modestly between 31.8% and 32.9%, while guanine (G) exhibited minimal fluctuations ranging from 12.2% to 12.3%. These findings suggest that the nucleotide structure is highly conserved across all growth conditions, despite minor differences.

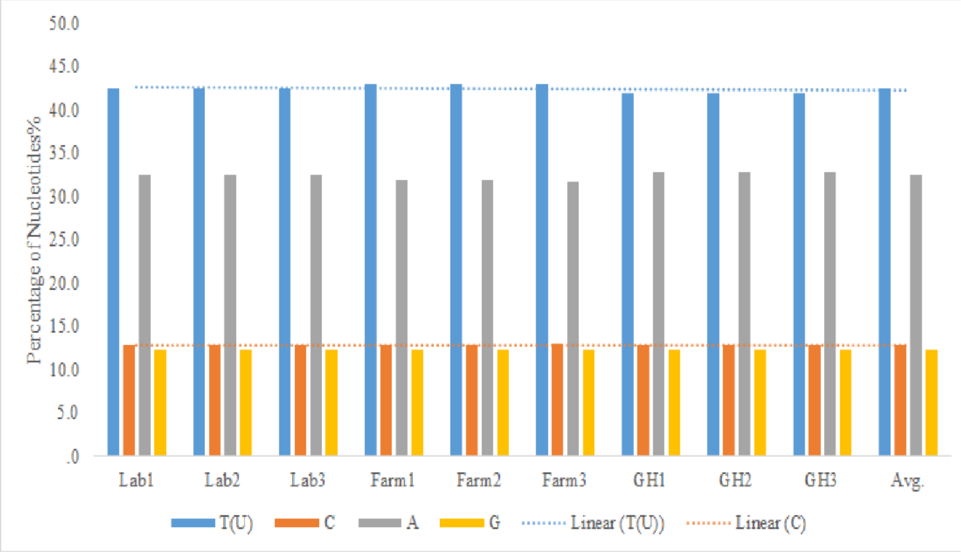

Figure 2. Mean nucleotide composition of a 632 bp COI fragment in *Tetranychus urticae* populations.

Population distance analysis

The genetic distance analysis of *T. urticae* populations reveals complete genetic uniformity within laboratory samples (0.0000) and minimal variation among greenhouse populations (0.0008 to 0.0010). Farm populations exhibit considerably higher genetic diversity, with distances ranging from 0.0201 to 0.0399. Genetic distances between laboratory and farm populations vary from moderate to high (0.0264 to 0.0929), while distances between greenhouse and farm populations fall within a moderate range (0.0132 to 0.0486). Laboratory and greenhouse groups are genetically closer to each other (0.0099 to 0.0128). These findings suggest that controlled environments limit genetic diversity, whereas farm populations maintain greater variability likely due to environmental heterogeneity and gene flow (Table 2).

Table 2. Pairwise genetic distances among *T. urticae* populations calculated using MEGA software.

	Lab1	Lab2	Lab3	Farm1	Farm2	Farm3	GH1	GH2	GH3
Lab1									
Lab2	0.0000								
Lab3	0.0000	0.0000							
Farm1	0.0264	0.0264	0.0264						
Farm2	0.0678	0.0678	0.0678	0.0201					
Farm3	0.0929	0.0929	0.0929	0.0399	0.0290				
GH1	0.0099	0.0099	0.0099	0.0162	0.0132	0.0486			
GH2	0.0099	0.0099	0.0099	0.0162	0.0132	0.0486	0.0009		
GH3	0.0128	0.0128	0.0128	0.0100	0.0100	0.0299	0.0010	0.0008	

Evolutionary Divergence

Among the *T. urticae* populations, Farm2, Farm3, and one greenhouse population exhibited distinct haplotypes and were clearly distinguished within their clade (Fig. 3). This haplotype differentiation likely results from localized selective pressures, specific environmental conditions, or restricted gene flow. This phylogenetic pattern not only confirms species-level divergence but also highlights the importance of examining intraspecific genetic diversity, particularly in *T. urticae*, as it can significantly influence the success of integrated pest management programs, including the selection of appropriate biological control agents.

Analysis of 632-nucleotide sequences for **T. urticae**

Analysis of three *T. urticae* populations using 632-nucleotide sequences of the mitochondrial COI gene revealed four distinct haplotypes (Fig. 4). A single haplotype (H1) was shared among all three populations (farm, greenhouse, and laboratory), indicating a common genetic origin. The farm population harbored two unique haplotypes (H2 and H3) absent in the other groups, whereas the greenhouse population contained one unique haplotype (H4). In contrast, the laboratory population exhibited only the shared haplotype, demonstrating complete genetic uniformity. Haplotype diversity (Hd) differed markedly among populations: the laboratory population showed no diversity (Hd = 0.000), reflecting full genetic uniformity under controlled conditions, while the greenhouse population exhibited very low diversity (Hd = 0.009), likely due to the dominance of a single haplotype and restricted gene flow within the enclosed environment. The farm population displayed the highest genetic diversity, with two unique haplotypes and Hd = 0.699, probably resulting from gene flow from wild populations, natural selection, and heterogeneous farming practices. These findings emphasize that both rearing conditions and population origin play critical roles in shaping the genetic structure of two-spotted spider mite populations and have important implications for pest management strategies.

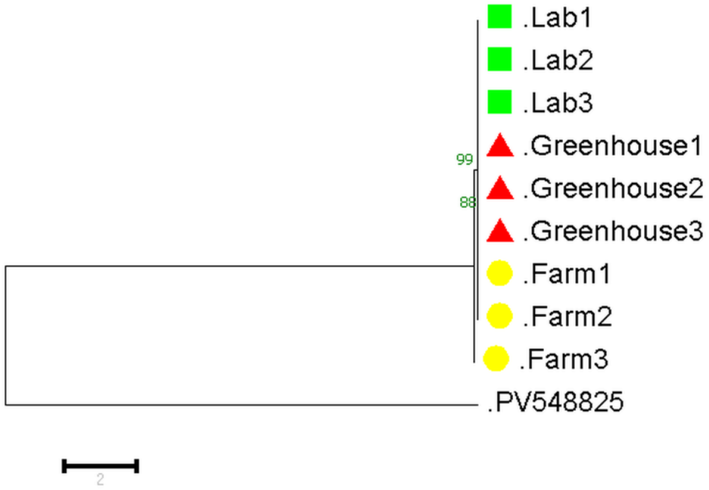

Figure 3. Phylogenetic relationships between populations of *T. urticae*.

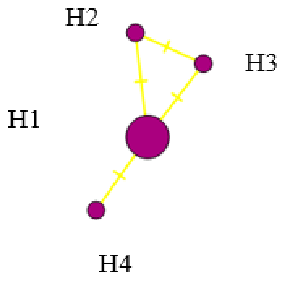

Figure 4. Haplotype network using popART software obtained for *T. urticae* – Each circle denotes a unique haplotype, and its size reflects the frequency of individuals associated with that haplotype across the sampled populations.

ISSR-Based Genetic Diversity in T. urticae

The genetic diversity indices calculated for the laboratory, greenhouse, and farm populations show clear differences that align with their environmental conditions and management practices. The laboratory population exhibited no genetic variation, with only one allele detected. The effective number of alleles was equal to one. Shannon's information index, expected heterozygosity, and unbiased expected heterozygosity were all zero, indicating a complete lack of genetic diversity. This is likely due to clonal reproduction or population bottlenecks under controlled conditions. The greenhouse population showed low genetic diversity, with two alleles identified and an effective allele number slightly above one. Diversity indices were low, reflecting limited polymorphism. This pattern suggests restricted gene flow and selective pressures in the semi-controlled greenhouse environment. The farm population exhibited significantly higher genetic diversity, with four alleles observed and a substantially higher effective number of alleles. This greater diversity is likely maintained by environmental variability, gene flow, and diverse selective pressures typical of open agricultural settings. Overall, the combined data from all populations indicate an intermediate level of genetic diversity, reflecting the influence of population origin and rearing environment (Table 3, Fig. 5).

Table 3. Genetic diversity indices of *Tetranychus urticae* populations based on ISSR marker.

Pop	Na	Ne	I	He	UHe
Laboratory	1.0	1.00	0.000	0.000	0.000
Greenhouse	2.0	1.20	0.010	0.009	0.010
Farm	4.0	2.50	0.700	0.690	0.695
Total	2.3	1.57	0.236	0.233	0.235

Na = Number of observed alleles or haplotypes; Ne = Effective number of alleles, accounting for allele frequencies; I = Shannon's information index, indicating genetic diversity; He = Expected heterozygosity, probability of heterozygous genotype; UHe = Unbiased expected heterozygosity, corrected for small sample sizes.

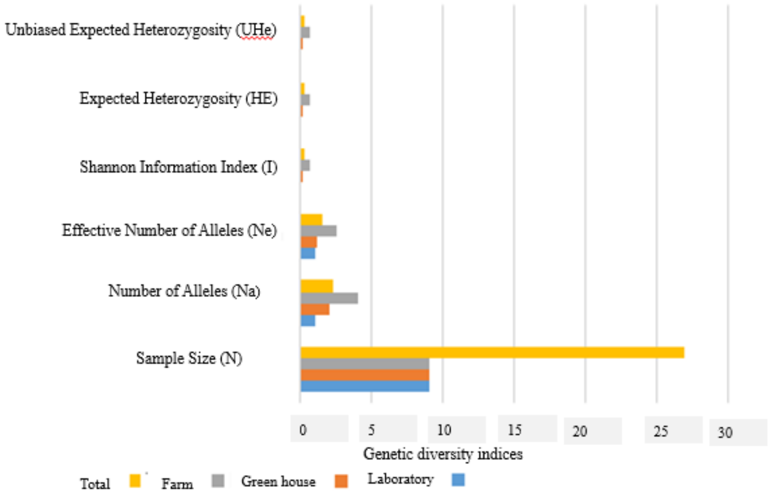

Figure 5. Genetic diversity indices of *T. urticae* based on ISSR markers.

PCoA-based genetic analysis of **T. urticae**

Principal Coordinates Analysis (PCoA) reveals clear genetic differentiation among laboratory, greenhouse, and farm populations based on ISSR data (Fig. 6). Laboratory samples form a distinct cluster, indicating reduced genetic diversity, likely due to inbreeding and prolonged isolation. Greenhouse individuals cluster closely together, suggesting moderate diversity and limited gene flow under semi-controlled conditions. In contrast, farm samples are more widely dispersed, reflecting greater genetic variability influenced by natural gene flow and environmental heterogeneity. The combined results from the phylogenetic tree and Principal Coordinates Analysis (PCoA) provide consistent and reliable evidence of genetic differentiation among *T. urticae* populations from laboratory, greenhouse, and field environments. Both analytical approaches reveal a clear genetic structure influenced by the degree of environmental control and gene flow.

Laboratory populations (Lab1 to Lab3) formed a distinct and separate cluster in both analyses. In the phylogenetic tree, they appeared as an independent clade, while in the PCoA plot, they were clearly isolated from other populations. This pattern reflects reduced genetic diversity due to inbreeding and isolation in controlled laboratory conditions. Although these populations are useful for controlled studies, they may not accurately represent the genetic diversity found in natural populations, so their use in biological control research should be approached with caution.

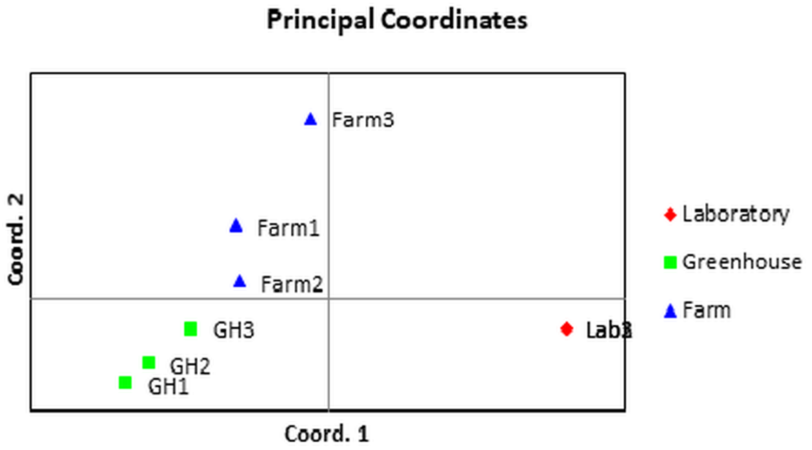

Figure 6. PCoA of *T. urticae* populations using ISSR data.

Greenhouse populations (GH1 to GH3) showed moderate genetic diversity and clustered closely in both analyses. This suggests that the semi-controlled greenhouse environment allows for limited but ongoing gene flow. Their close grouping in both the phylogenetic tree and PCoA plot indicates occasional introduction of new individuals or restricted gene exchange, maintaining some level of variability. In contrast, field populations (Farm 1 to Farm 3) exhibited the highest genetic diversity and were clearly distinct from other groups, especially Farm 2 and Farm 3. This reflects the influence of local selective pressures, environmental variability, and broader gene flow in natural environments. Such high diversity may increase adaptability but also complicate pest management efforts.

The strong agreement between the phylogenetic and PCoA results not only enhances the credibility of the findings but also highlights the importance of assessing intraspecific genetic diversity when designing integrated pest management (IPM) programs. Populations with different genetic backgrounds may respond differently to biological control agents, pesticides, or environmental changes. Therefore, understanding population structure is essential for selecting effective and sustainable control measures.

DISCUSSION

Regarding tetranychid mites, DNA-based identification offers several benefits over conventional morphological techniques. It is faster, as it allows the use of specimens from any developmental stage, eliminating the need for the lengthy and potentially hazardous process of rearing spider mites. (Marrelli *et al.* 2005). Numerous investigations have utilized molecular markers, especially the COI and ITS gene regions, to identify spider mite species (Ben-David *et al.* 2007; Ros and Breeuwer 2007; Osakabe *et al.* 2008; Hada *et al.* 2016). In some cases, researchers have applied three different molecular markers at the same time to distinguish species belonging to the Tetranychidae family. However, despite the significant importance of these mites in Iran, genetic studies and sequence records in this field have been very limited, and relatively little attention has been given to the topic. In this study, the genetic structure of *T. urticae* varied significantly across laboratory, greenhouse, and farm environments. Laboratory populations exhibited the lowest genetic diversity, likely due to small founding populations, controlled environmental conditions, and limited selective pressures. The stable and isolated nature of laboratory settings restricts gene flow and allows genetic drift to rapidly fix alleles, leading to increased genetic uniformity. Greenhouse populations showed slightly higher genetic diversity than laboratory populations but still maintained relatively low variation and close genetic similarity. This pattern likely results from semi-controlled conditions, restricted migration, small population sizes, and repeated pesticide applications that favor resistant genotypes and reduce overall diversity. In contrast, farm populations displayed significantly greater genetic diversity. While some farm populations were genetically similar, others, such as Farm3, exhibited pronounced divergence. This increased diversity is probably driven by gene flow from wild or unmanaged populations, variable pesticide use, larger and more dynamic population sizes, and heterogeneous environmental pressures. These findings underscore the critical role of agricultural practices and ecological complexity in maintaining or enhancing genetic diversity. Only four COI haplotypes were identified across the three *T. urticae* populations in this study, compared to 24 haplotypes reported by İnak (2021). This discrepancy likely reflects differences in sampling scope and habitat types. While the present study focused on confined and managed environments, İnak's work included wild and geographically diverse populations, which naturally harbor greater genetic variation due to wider environmental exposure and gene flow. Differences in sample size and sequencing depth may also contribute to the difference.

In this study, two distinct COI haplotypes in *T. urticae* were identified in the farm populations, while an average of four alleles was detected across three ISSR loci (Tu1, Tu11, and Tu35b). This difference reflects the nature and genomic scope of the molecular markers used. The mitochondrial COI gene, which traces maternal lineage, is more conserved and typically reveals lower levels of genetic variation. In contrast, ISSR markers target multiple regions across the nuclear genome, allowing for the detection of broader genetic diversity. Therefore, the combined use of both marker types offers a more comprehensive understanding of population genetic structure. While COI primarily captures mitochondrial lineage history, ISSR reveals variation in the nuclear genome and can uncover subtle genetic differences among populations that mitochondrial markers alone may miss. For effective management of tetranychid mites in Iran, integrating genetic and molecular tools into pest control programs is essential. Traditional methods based on morphological identification are often slow and prone to errors, making the development and standardization of DNA-based techniques crucial for rapidly and accurately assessing the genetic diversity of these species. Given the current limitations in sampling and available data, future research should focus on expanding sampling across broader geographic regions and diverse natural and agricultural habitats to provide a more comprehensive understanding of the genetic variation among tetranychid mite populations in Iran. Additionally, establishing a national genetic database and developing rapid DNA-based diagnostic kits could significantly enhance pest management strategies.

CONCLUSION

Recent research has clearly shown that molecular techniques have transformed the way tetranychid mites are managed. DNA-based approaches have largely replaced traditional morphological identification methods, which were often slow and prone to errors. These advancements are especially important given the diverse mite fauna in Iran and the possibility of unknown native or invasive species. Major outcomes of this scientific shift include the application of molecular tools for assessing genetic diversity and population structure, the development of genetic monitoring for resistance, the implementation of precise control measures with limited environmental effects, and the formulation of sustainable strategies to preserve agricultural ecosystem health. National adoption of genetic monitoring provides numerous benefits such as precise species detection, early recognition of resistance emergence, implementation of localized control plans, and considerable reduction in chemical pesticide use. Future efforts should prioritize establishing a comprehensive genetic database for mites in Iran, designing quick molecular diagnostic tools, combining genomic information with advanced computational methods for predicting population trends, and improving targeted biological control techniques.

Author contributions: Methodology: F.A.: Conceptualization, Sampling, Collection and preparation of specimens, DNA extraction, PCR and data curation, Writing – original draft, Writing – review and editing; M.K.: Assistance in Materials and Methods, PCR optimization, Data analysis; M.A., M.K.: Assistance in sample collection and species identification. All authors have read and agreed to the published version of the manuscript.

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Ethics approval and consent to participate: This study only included arthropod material, and all required ethical guidelines for the treatment and use of animals were strictly adhered to in accordance with international, national, and institutional regulations. No human participants were involved in any studies conducted by the authors for this article.

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ساختار جمعیتی و تنوع ژنتیکی کنهٔ تارتن دو لکه‌ای *Tetranychus urticae* (Acari: Tetranychidae) در زیستگاه‌های مختلف بر پایه نشانگرهای ISSR و *COI*

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دبیر تخصصی
ع. صبوری

چکیده

در این پژوهش، ساختار جمعیتی و تنوع‌ژنتیکی کنه تارتن دو لکه‌ای، *Tetranychus urticae* Koch (Acari: Tetranychidae) در سه محیط متفاوت شامل مزرعه، گلخانه و آزمایشگاه بررسی شد. تحلیل قطعه‌ای ۶۳۲ نوکلئوتیدی از ژن میتوکندریایی *COI*، چهار هاپلوتیپ متمایز را شناسایی کرد؛ یکی از این هاپلوتیپ‌ها در تمامی جمعیت‌ها حضور داشت که نشان‌دهنده‌ی منشأ ژنتیکی مشترک آن‌هاست. در میان جمعیت‌های مورد بررسی، جمعیت مزرعه بیشترین میزان تنوع هاپلوتیپی را نشان داد (Hd = 0.699)، در حالی‌که جمعیت گلخانه کمترین تنوع را داشت و جمعیت آزمایشگاهی از یکنواختی کاملی برخوردار بود. بررسی فاصله ژنتیکی نیز این تفاوت‌ها را تأیید کرد و نشان داد که میان جمعیت مزرعه و جمعیت‌های یکنواخت‌تر گلخانه و آزمایشگاه فاصله ژنتیکی معنی‌داری وجود دارد. نتایج حاصل از نشانگرهای ISSR این الگوها را پشتیبانی کرد؛ به‌طوری‌که جمعیت مزرعه بیشترین تعداد آلل (۴ آلل) و آلل‌های مؤثر، و همچنین مقادیر بیشتر شاخص اطلاعات شانون و هتروزیگوسیتی مورد انتظار را داشت. تحلیل مختصات اصلی (PCoA) نیز به‌روشنی بیانگر یک الگوی خوشه‌بندی ژنتیکی مشخص بود: نمونه‌های آزمایشگاهی، خوشه‌ای فشرده و مجزا تشکیل دادند، نمونه‌های گلخانه خوشه‌ای پراکندگی متوسط و نمونه‌های مزرعه پراکندگی گسترده‌ای داشتند. این الگوها نشان می‌دهد که شرایط پرورشی تأثیر چشمگیری بر ساختار ژنتیکی *T. urticae* دارند؛ شرایط باز مزرعه تنوع‌ژنتیکی را تقویت، اما محیط‌های کنترل‌شده به کاهش این تنوع می‌انجامند. این یافته‌ها برای درک سازگاری آفت و بهینه‌سازی راهبردهای مدیریت تلفیقی آفات (IPM) اهمیت دارند.

واژگان کلیدی: تغییرات آللی، هاپلوتیپ، ژنتیک جمعیت، تحلیل مختصات اصلی، کنهٔ تارتن

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