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Genetic Diversity and Connectivity in Iranian Populations of *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae)

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ABSTRACT

Understanding pest population genetics is vital for management strategies. This study examines the genetic diversity and structure of *Plutella xylostella* (Linnaeus, 1758) a major global pest, across eight rapeseed-producing provinces in Iran, where comprehensive national-scale data are currently lacking. A total of 72 individuals from 24 sampling sites in eight major rapeseed (*Brassica napus*) producing provinces were analyzed based on a 509-bp fragment of the cytochrome oxidase I (*COI*) gene. A total of 20 distinct haplotypes were identified, indicating high haplotype (Hd= 0.975) and nucleotide diversity (π= 0.01276). Analysis of molecular variance (AMOVA) revealed that the majority of genetic variation was distributed within populations (56.9%), while the remaining variation was attributed to differences among populations within provinces (14.8%) and among provinces (28.3%). The relatively high fixation index (F_{ST}= 0.43) indicated significant genetic differentiation among populations. Isolation-by-distance analysis showed a weak correlation between genetic and geographic distances (R^2= 0.126), implying that factors other than simple geographic distance may influence population structure. However, because mitochondrial markers cannot distinguish between natural dispersal and human-mediated movement, inferences regarding gene flow should be made cautiously. Overall, this study provides valuable insights into the genetic diversity and population structure of *P. xylostella* in Iran and highlights the need for future studies incorporating nuclear markers or genome-wide approaches to better understand dispersal dynamics and resistance evolution.

Keywords: *COI* gene, Gene flow, Haplotype diversity, Pest resistance, Population structure.

INTRODUCTION

The order Lepidoptera includes some of the most significant insect pests worldwide, many of which threaten food security and agricultural sustainability by damaging crops and horticultural

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plants (Talekar and Shelton, 1993; Zalucki *et al*., 2012).

The diamondback moth, *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae), is one of the most destructive pests of Brassicaceae crops globally. Its high reproductive capacity, wide dispersal ability, and rapid development of resistance to insecticides make it a major challenge for pest management (Talekar and Shelton, 1993; Furlong *et al*., 2013). Global economic losses caused by this pest, including yield reductions and control costs, are estimated to reach billions of US dollars annually (Zalucki *et al*., 2012).

In Iran, *P. xylostella* was first reported in 1938 by Afshar from the Caspian Sea coastal areas and regions around Tehran. It is now considered one of the most important pests of cabbage, canola, and other Brassicaceae crops (Nouri-Ganbalani *et al*., 2018). For instance, in 2022, infestations in Golestan Province one of Iran's main canola-producing regions caused severe damage to approximately 5,000 hectares of canola, requiring extensive chemical control measures. *P. xylostella* in Iran has developed significant resistance to insecticides. This resistance is primarily driven by multiple biochemical mechanisms, including glutathione S-transferases (GSTs), esterases, and cytochrome P450 monooxygenases (Sayani *et al*., 2019). Certain GST genes, especially GSTd4 and PxGSTe2, play a key role in this resistance. Iranian populations exhibit a multi-mechanistic resistance pattern (Sayani *et al*., 2019; Hasanshahi *et al*., 2013). This genetic complexity highlights the urgent need for integrated pest management (IPM) strategies, indicating that reliance on chemical control alone is no longer sufficient. Genetic studies on insecticide resistance provide critical insights for developing targeted and sustainable pest management approaches.

Molecular markers, particularly the mitochondrial cytochrome c oxidase subunit I (*COI*) gene, are essential tools for assessing genetic diversity and population structure in insect pests (Hebert *et al*., 2003, 2004; Abdolahadi *et al*., 2022; Saran and Genç, 2024; Valiyaparambil *et al*., 2024; Hosseini *et al*., 2025). *COI* sequences have been widely used to evaluate haplotype diversity in *P. xylostella*. They also provide information on genetic connectivity and population differentiation (Caprio and Tabashnik, 1992; Yang *et al*., 2013; Yang *et al*., 2015; Perry *et al*., 2013). Recent genomic advances in Lepidoptera, encompassing over 160,000 described species (Kawahara *et al*., 2019; Iang *et al*., 2024). Other markers, including allozymes, ISSRs, and microsatellites, have also been employed to study population structure (Endersby *et al*., 2006; Pichon *et al*., 2006; Juric *et al*., 2017). Investigating the genetic structure of *P. xylostella* populations in Iran can provide a crucial foundation for understanding their genetic diversity, dispersal patterns, and gene flow. Given the relatively small sample size per population and the use of only a mitochondrial marker, the results of this study should be interpreted with caution. Therefore, the main objective of this study is to analyze the genetic diversity and population structure of DBM across key Brassicaceae-growing regions in Iran. The insights gained from this research can inform the development of targeted and sustainable integrated pest management (IPM) strategies, complementing traditional control methods and improving the management of this economically important pest.

MATERIALS AND METHODS

Sample Collection

In this study, *P. xylostella* specimens were collected from eight provinces in Iran: Hamadan, Tehran, Isfahan, East Azerbaijan, Fars, South Khorasan, Khuzestan, and Mazandaran (Table 1). These provinces were selected to cover a range of climatic conditions, allowing us to examine the influence of environmental factors and geographic distance on genetic variation within populations. Sampling was conducted using two complementary methods: light traps and manual collection. Approximately 15 specimens were collected from each county. For molecular analyses, only adult individuals that were manually collected and exhibited clear diagnostic morphological features specifically, creamy-white spots on the wings forming a diamond-shaped band when the wings are closed were selected. Manual collection ensured that the specimens originated exclusively from the target host plant (*Brassica napus*), minimizing the possibility of contamination from alternative hosts. From these manually collected specimens, three individuals per county were chosen for molecular analysis, and each was analyzed in triplicate, resulting in a total of 72 individuals and 216 samples. All specimens were immediately preserved in 95% ethanol and stored at –20°C to maintain DNA integrity for sequencing. This careful collection and selection procedure ensures that the molecular data accurately reflect the true genetic composition of *P. xylostella* populations in Iran.

All samples and sequences (72 in total) were identified for each population and have been deposited in GenBank. All of these samples were included in the median-joining network and AMOVA analyses.

Table 1. Sampling locations of *P. xylostella* populations in Iran, including geographic coordinates, altitude, and climate type (Köppen–Geiger classification) (Kottek *et al*., 2006).

Province	**City**	**Latitude**	**Longitude**	**Height (m)**	**City Code**	**Climate (Köppen–Geiger)**
East Azerbaijan	Maragheh	37°24′39.98″ N	46°12′26.78″ E	1451	1	Cold semi-arid (BSk)
	Azar-shahr	37°44′48.11″ N	45°57′37.19″ E	1382	2	Cold semi-arid (BSk)
	Marand	38°27′35.83″ N	45°45′57.20″ E	1331	3	Cold semi-arid (BSk)
Isfahan	Shahin Shahr	32°52′57.89″ N	51°35′45.33″ E	1592	4	Cold semi-arid (BSk)
	Khansar	33°16′32.48″ N	50°18′46.08″ E	2215	5	Cold semi-arid (BSk)
	Shahreza	32°02′00.62″ N	51°53′37.17″ E	1833	6	Cold semi-arid (BSk)
Tehran	Varamin	35°20′02.40″ N	51°41′19.68″ E	920	7	Cold semi-arid (BSk)
	Damavand	35°41′12.48″ N	52°06′10.08″ E	1940	8	Cold semi-arid (BSk)
	Rey	35°35′35.52″ N	51°24′15.84″ E	1065	9	Cold semi-arid (BSk)
Hamedan	Kabudarahang	35°13′37.00″ N	48°44′31.22″ E	1591	10	Cold semi-arid (BSk)
	Malayer	34°18′02.13″ N	48°47′18.40″ E	1748	11	Cold semi-arid (BSk)
	Asadabad	34°46′47.88″ N	48°05′29.40″ E	1672	12	Cold semi-arid (BSk)
Mazandaran	Babol	36°33′23.29″ N	52°41′57.92″ E	42	13	Humid subtropical (Cfa)
	Kelardasht	36°31′22.33″ N	51°13′12.85″ E	94	14	Humid subtropical (Cfa)
	Chahardangeh	36°34′45.26″ N	53°01′56.33″ E	29	15	Humid subtropical (Cfa)
South Khorasan	Tabas	33°35′03.84″ N	56°54′44.64″ E	1454	16	Hot desert (BWh)
	Ferdows	34°00′07.20″ N	58°08′36.96″ E	1269	17	Cold semi-arid (BSk)
	Birjand	32°52′43.68″ N	59°09′31.68″ E	661	18	Cold semi-arid (BSk)
Khuzestan	Shush	33°12′07.20″ N	48°14′11.40″ E	70	19	Hot desert (BWh)
	Dezful	32°23′21.11″ N	48°21′57.60″ E	2	20	Hot desert (BWh)
	Ramhormoz	31°16′49.44″ N	49°34′06.24″ E	166	21	Hot desert (BWh)
Fars	Kazerun	29°37′27.84″ N	51°36′47.52″ E	834	22	Hot semi-arid (BSh)
	Sarvestan	29°16′17.76″ N	53°11′49.92″ E	1380	23	Cold semi-arid (BSk)
	Fasa	28°57′43.30″ N	53°39′54.72″ E	1350	24	Cold semi-arid (BSk)

DNA Extraction

Genomic DNA was extracted from the abdomens of adult *P. xylostella* using the TOP General Genomic DNA Purification Kit (Topaz Gene Company, Tehran, Iran), following the manufacturer's instructions with minor modifications. Additional homogenization steps were incorporated to ensure thorough tissue disruption, and an extra washing step was performed to improve DNA purity. To ensure robust genetic analyses, 24 populations were included in the study, with three individuals sampled from each population. Each individual was analyzed in triplicate, resulting in a total of 72 individuals and 216 samples. The quantity and quality of the extracted DNA were assessed prior to downstream analyses. DNA integrity was evaluated using 0.7% agarose gel electrophoresis, while PCR products were analyzed on a 2% agarose gel. Extracted DNA was stored at –20°C until further molecular processing.

PCR Amplification and Sequencing

A fragment of the mitochondrial cytochrome oxidase subunit I (*COI*) gene was amplified using primers designed based on (Yukuhiro *et al*., 2002). The forward primer sequence was 5'-AAATTTACAATTTATCGCTTAAATCTCAGCC-3', and the reverse primer sequence was 5'-CCTCTTTCTTGTGATAATAATATGGAAATTATACC-3'. PCR reactions were performed in a total volume of 20 µL, consisting of 12.5 µL PCR Master Mix, 30 ng genomic DNA, 2 µL nuclease-free water, and 1 µL of each primer. Cycling conditions included an initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 52°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 5 min. PCR products were visualized on a 2% agarose gel containing 1 µg/mL ethidium bromide (Barbosa *et al*., 2014). Products showing a single clear band were purified once in the laboratory and submitted to a commercial sequencing facility, where a second purification step was performed prior to bidirectional Sanger sequencing. All sequences obtained in this study were deposited in GenBank under accession numbers PQ012996–PQ013001, PQ032573–PQ032578, PX783248–PX783259, PX783260- PX783271 and PZ017399–PZ017434.

Sequence Alignment and Genetic Analyses

Sequence alignment and subsequent genetic analyses were performed using MEGA version 11.0.10 with the Clustal W algorithm (Kumar *et al*., 2016). Phylogenetic relationships were inferred using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura, 1980), with 1,000 bootstrap replicates to assess node support. Nucleotide composition and pairwise genetic distances were also calculated within MEGA. Population genetic parameters, including haplotype diversity, nucleotide diversity, and neutrality tests, were estimated using DnaSP version 5 (Librado and Rozas, 2009). Haplotype relationships were visualized through median-joining network analysis in PopART version 1.7.2 (Leigh and Bryant, 2015). The correlation between genetic differentiation and geographic distance was assessed using a Mantel test in GenAlEx version 6.5 (Peakall and Smouse, 2012) with 999 permutations. Further population structure and genetic differentiation were evaluated via Analysis of Molecular Variance (AMOVA) and F_{ST} calculations using Arlequin version 3.5.2.2 (Excoffier and Lischer, 2010). These analyses provided a comprehensive understanding of the genetic structure and evolutionary relationships among the studied populations.

RESULTS

Analysis of nucleotide composition revealed that thymine (38.8%) and adenine (30.5%) were the most abundant nucleotides, while cytosine (15.4%) and guanine (15.3%) were less frequent. The 509-bp COI fragment exhibited a pronounced A+T bias (69.3%), which is consistent with typical insect mitochondrial DNA and previous studies on *P. xylostella*. Of the 509 analyzed sites, 484 were conserved, whereas 25 sites were variable, giving rise to 20 distinct haplotypes. This resulted in a high haplotype diversity (Hd = 0.975), indicating substantial genetic variation among the sampled *P. xylostella* populations across different regions of Iran.

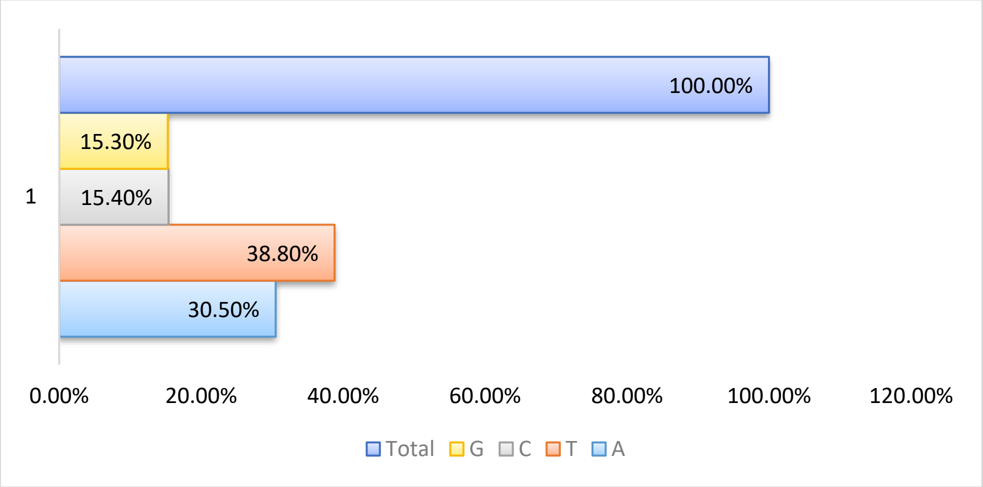

Figure 1. Average nucleotide composition of a 509 bp *COI* gene segment in 24 studied populations of *P. xylostella* in Iran.

Table 2. Distribution of haplotypes in different populations of *P. xylostella* across different provinces of Iran.

Haplotype	East Azerbaijan	Mazandaran	Hamedan	Khuzestan	Isfahan	South Khorasan	Tehran	Fars	Total
H1	1	1	1	1	1		1	1	7
H2	1	1	1						3
H3		1							1
H4	1	1		1					3
H5	1	1							2
H6		1		1					2
H7		1		1					2
H8	1	1							2
H9			1	1					2
H10	1	1	1						3
H11			1	1					2
H12						1			1
H13		1	1	1					3
H14	1		1						2
H15				1	1				2
H16			1		1				2
H17	1				1				2
H18					1		1		2
H19							1	1	2
H20				1	1				2
Total	8	10	8	9	6	1	3	2	20

Note: The total value (20) represents the number of unique haplotypes identified across the entire dataset and does not correspond to the sum of haplotypes across individual provinces.

A total of 20 distinct haplotypes (H1–H20) were identified among the sampled populations of *P. xylostella* (Table 2). Haplotype H1 was the most frequent and showed the widest geographic distribution, occurring in seven of the eight provinces. In contrast, haplotype H12 was detected exclusively in the South Khorasan population. Mazandaran Province showed the highest haplotype diversity among the sampled regions, followed by Khuzestan and East Azerbaijan (Table 2). Overall, these results indicate a heterogeneous genetic structure across the Iranian *P. xylostella* populations. In this study, nucleotide diversity (π) was assessed in *P. xylostella* populations from eight provinces in Iran (Table 3). The overall nucleotide diversity across all provinces was relatively low to moderate ($\pi = 0.01276$), while haplotype diversity was high. Mazandaran exhibited the highest nucleotide diversity ($\pi = 0.00746$), whereas South Khorasan and Fars showed the lowest values ($\pi = 0.00149$ and $\pi = 0.00224$, respectively). These differences may reflect regional variation in population history, demographic processes, or sampling effects, rather than definitive genetic isolation. Given the use of a single mitochondrial marker, population differentiation was evaluated using AMOVA rather than pairwise comparisons.

Table 3. Genetic diversity analysis of eight *P. xylostella* populations based on mitochondrial gene sequences.

Province	The total number of populations	Sample Size per Population (N)	Nucleotide Diversity (π)	Polymorphic Sites (S)
Tehran	3	3	0.00235	3
East Azerbaijan	3	3	0.00572	8
Mazandaran	3	3	0.00746	10
Khuzestan	3	3	0.00671	9
Hamedan	3	3	0.00549	6
South Khorasan	3	3	0.00149	2
Isfahan	3	3	0.00449	5
Fars	3	3	0.00224	3
Total	24	72	0.01276	25

N= number of populations, Hd= haplotype diversity, S= Number of segregating sites. Total π and S values represent calculations based on the entire dataset of 24 sequences and are not derived by summing or averaging the values of individual provinces.

These differences are likely due to population history, environmental variation, or sampling effects and should not be interpreted as definitive evidence of genetic isolation. The COI-based haplotype network illustrates the relationships among populations, with the dominant haplotype H1 found across multiple regions, reflecting its wide distribution. The absence of clear geographic clustering in the network may indicate the species high dispersal ability and potential human-mediated.

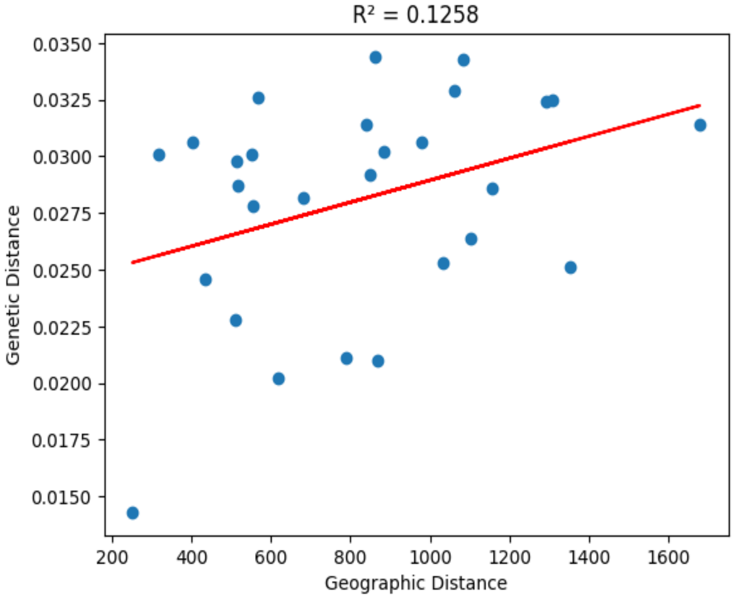

Figure 2. Weak positive correlation between geographic distance and genetic distance indicates isolation by distance in Iranian populations of *P. xylostella.*

This study examined genetic distances among populations from various Iranian provinces using genetic data. The results showed the smallest genetic distance between Mazandaran and East Azerbaijan (0.0197), and the largest between South Khorasan and Mazandaran (0.0373). Although there was a weak positive correlation between geographic and genetic distances ($R^2 = 0.1258$), which explained only 12.6% of the variance, this suggests that other factors, such as gene flow, evolutionary history, and natural selection, may also play a role in shaping genetic variation. These findings emphasize the complexity of the genetic structure of these populations and suggest that genetic distances may be influenced by various biological and evolutionary factors, rather than exhibiting a direct linear relationship with geographic distances.

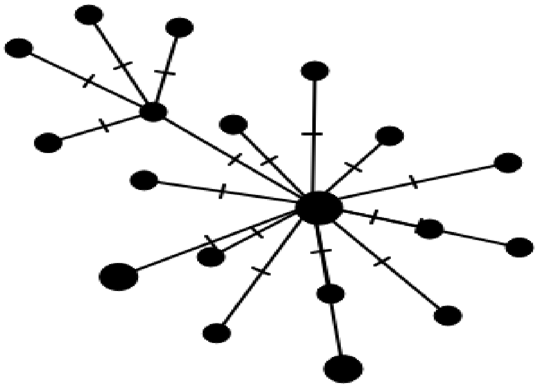

Figure 3. Median-joining haplotype network of unique mitochondrial COI haplotypes of *P. xylostella* from Iran. Each circle represents a distinct haplotype, with its size proportional to haplotype frequency.

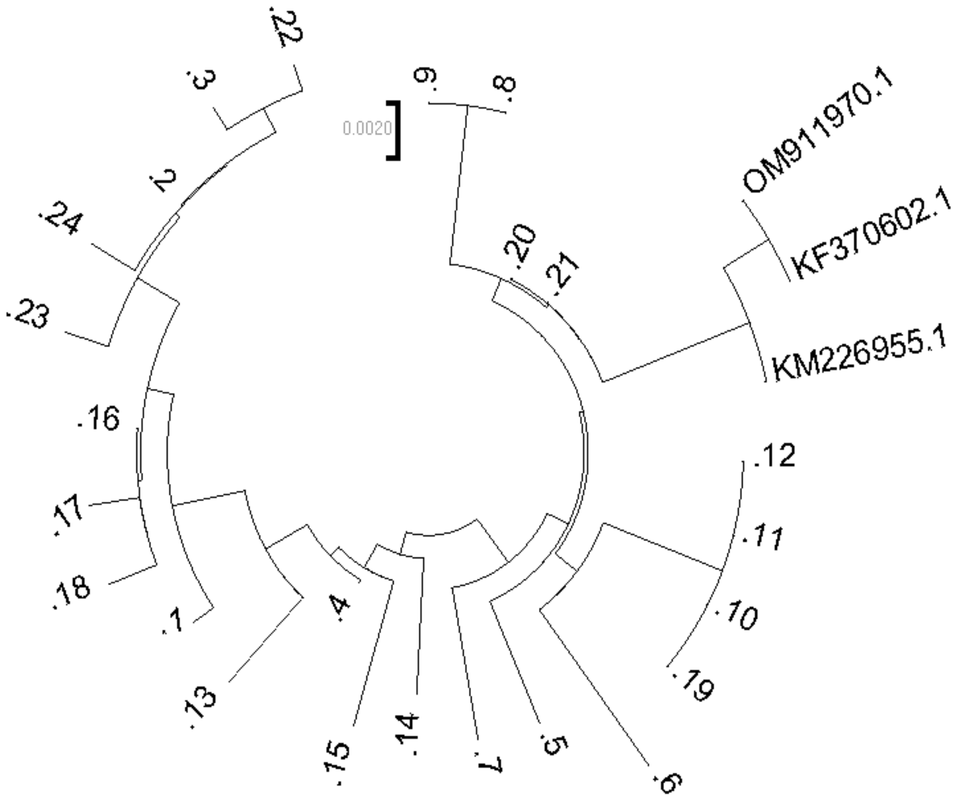

Figure 4. Maximum Likelihood tree constructed using 24 haplotypes of the *COI* gene among 24 populations of *P. xylostella.* The codes corresponding to the cities are provided in Table 1.

The phylogenetic tree constructed based on 24 *COI* sequences showed a shallow genetic structure, with samples distributed across multiple branches rather than forming well-supported, distinct clades. Sequences from different provinces were interspersed throughout the tree, indicating a lack of clear geographic clustering. This pattern is consistent with the low nucleotide divergence observed among haplotypes and the results of the haplotype network analysis. This pattern may also be influenced by the relatively small sample size per province, which may reduce the power to detect well-supported phylogenetic structure and contribute to the dispersed placement of sequences in the tree.

Table 4. Analysis of Molecular Variance (AMOVA) Revealing Genetic Structure across Geographic Hierarchies.

Source of Variation	df	Sum of Squares	Variance Components	% of Total Variation	Fixation Index	p-value
Among Provinces	7	27.10	1.05	28.3%	F_{CT}= 0.28	0.002
Among Populations/Within Provinces	16	18.00	0.55	14.8%	F_{SC}= *0.21*	0.01
Within Populations	48	41.00	2.13	56.9%	F_{ST} = 0.43	< 0.001
Total	71	86.10	—	100%	—	—

The AMOVA analysis revealed that the majority of genetic variation is found within populations (56.9%), indicating a high level of haplotypic diversity at the local population level. A significant proportion of the total variation (28.3%) was attributed to differences among provinces (F_{CT} = 0.28, p = 0.002), reflecting a pronounced geographic genetic structure at the provincial scale. Additionally, genetic differentiation among populations within provinces accounted for 14.8% of the variation (F_{SC} = 0.21, p = 0.01), suggesting notable local-level genetic differentiation possibly due to uneven gene flow or distinct demographic histories. The overall fixation index F_{ST} = 0.43, $p < 0.001$) further supports the presence of substantial genetic structuring among populations. These findings are consistent with other analyses in this study, including phylogenetic trees and haplotype networks, which collectively demonstrate extensive haplotypic diversity and genetic differentiation across the studied regions.

DISCUSSION

In this study, the genetic diversity and population structure of *P. xylostella* in Iran were investigated using the mitochondrial COI marker. Our results showed that Iranian populations exhibited relatively high haplotype diversity but low nucleotide diversity, a pattern consistent with reports from other migratory populations worldwide (Wei *et al*., 2013; Li *et al*., 2006; Yang *et al*., 2015). This suggests that most genetic variation occurs at the haplotype level and likely reflects the species demographic history and dispersal capacity. Although the present study was restricted to populations collected exclusively from (*Brassica napus*), the role of various host plant species likely plays a significant part in shaping the high haplotype diversity observed in *P. xylostella*. Different host plants can impose varying selective pressures or influence dispersal patterns, potentially leading to genetic differentiation. Therefore, we suggest that future studies should include samples from diverse host plants to fully elucidate the impact of host species on the population structure and genetic diversity of this pest. A total of 20 distinct haplotypes (H1–H20) were identified. Haplotype H1 was the most common and widespread, occurring in seven provinces, whereas H12 was restricted to South Khorasan. This pattern may indicate local differences in population distribution and dispersal. However, given the limited sample size and reliance on a single mitochondrial marker, definitive conclusions about population differentiation should be made with caution. AMOVA analysis indicated that a substantial portion of the genetic variation occurred at the haplotype level among populations, with an F_{ST} of 0.43 reflecting moderate genetic differentiation between provinces, although gene flow is still evident. These findings are in line with previous studies in China and Turkey, which reported significant gene flow and widespread distribution of dominant haplotypes (Saran and Genç, 2024; Wei *et al*., 2013). The weak correlation between genetic and geographic distances ($R^2 = 0.126$) suggests that non-geographic factors, such as short-distance dispersal, human-mediated movement, and historical demographic events, likely contribute to population structure. This observation aligns with global reports showing that even geographically close populations can maintain high genetic diversity while still experiencing gene flow (Caprio and Tabashnik, 1992; Wei *et al*., 2013).

CONCLUSIONS

Overall, the combination of high haplotype diversity, low nucleotide diversity, and weak geographic correlation indicates that *P. xylostella* populations in Iran are shaped by a complex interplay of demographic history, gene flow, and limited dispersal. However, limitations related to sample size and the use of a single mitochondrial marker restrict the precision of these interpretations. Future studies employing nuclear markers or genome-wide analyses are needed to clarify migration patterns, gene flow, and population structure more accurately, providing a stronger foundation for region-specific integrated pest management (IPM) strategies.

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تنوع ژنتیکی و ارتباط در جمعیت‌های ایرانی شب پره پشت الماسی کلم (*Plutella xylostella*) (Linnaeus, 1758) (Lepidoptera: Plutellidae)

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چکیده

درک ژنتیک جمعیت آفات برای استراتژی‌های مدیریتی حیاتی است. این مطالعه تنوع ژنتیکی و ساختار *Plutella xylostella* (Linnaeus, 1758) یک آفت مهم جهانی را در هشت استان تولیدکننده کلزا در ایران، که در حال حاضر داده‌های جامع در مقیاس ملی در آنها وجود ندارد، بررسی می‌کند. در مجموع ۷۲ فرد از ۲۴ محل نمونه‌برداری در هشت استان اصلی تولیدکننده کلزا (*Brassica napus*) بر اساس قطعه ۵۰۹ جفت بازی از ژن سیتوکروم اکسیداز I (COI) مورد تجزیه و تحلیل قرار گرفتند. در مجموع ۲۰ هاپلوتیپ متمایز شناسایی شد که نشان‌دهنده تنوع هاپلوتیپی بالا (Hd= 0.975) و تنوع نوکلئوتیدی بالا ($\pi= 0.01276$) است. تجزیه و تحلیل واریانس مولکولی (AMOVA) نشان داد که اکثر تنوع ژنتیکی در داخل جمعیت‌ها (٪۵۶.۹) توزیع شده است، در حالی که تنوع باقی مانده به تفاوت‌های بین جمعیت‌ها در داخل استان‌ها (٪۱۴.۸) و بین استان‌ها (٪۲۸.۳) نسبت داده شد. شاخص تثبیت نسبتاً بالا (FST= 0.43) نشان‌دهنده تمایز ژنتیکی قابل توجه بین جمعیت‌ها بود. تجزیه و تحلیل جداسازی بر اساس فاصله، همبستگی ضعیفی بین فواصل ژنتیکی و جغرافیایی نشان داد ($R^2= 0.126$)، که نشان می‌دهد عواملی غیر از فاصله جغرافیایی ساده ممکن است بر ساختار جمعیت تأثیر بگذارند. با این حال، از آنجا که نشانگرهای میتوکندریایی نمی‌توانند بین پراکندگی طبیعی و حرکت با واسطه انسان تمایز قائل شوند، استنتاج در مورد جریان ژن باید با احتیاط انجام شود. به طور کلی، این مطالعه بینش‌های ارزشمندی در مورد تنوع ژنتیکی و ساختار جمعیت *P. xylostella* در ایران ارائه می‌دهد و نیاز به مطالعات آینده با استفاده از نشانگرهای هسته‌ای یا رویکردهای ژنومی را برای درک بهتر پویایی پراکندگی و تکامل مقاومت برجسته می‌کند.