ÇOMÜ Zir. Fak. Derg. (COMU J. Agric. Fac.)
2022: 10 (1): 211–221
ISSN: 2147–8384 / e-ISSN: 2564–6826
doi: 10.33202/comuagri.1054611

Araştırma Makalesi/Reserach Article

Genetic Diversity of Cotton Bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae) Population in Çanakkale

Seda Yücel[1] **Hanife Yandayan Genç***[2]

[1]School of Graduate Studies, Department of Agricultural Biotechnology, Çanakkale Onsekiz Mart University, Turkey
[2]Department of Agricultural Biotechnology, Faculty of Agriculture, Çanakkale Onsekiz Mart University, Çanakkale, Turkey
*Corresponding author: hgenc@comu.edu.tr[2]

Received Date: 07.01.2022 Accepted Date: 31.03.2022

Abstract

The cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is a commonly known polyphagous pest in agricultural areas all over the world. It has been a major pest causing significant damages by reducing tomato fruit production and quality since 1913 in Turkey. The objective of the study was to evaluate genetic diversity and phylogenetic relationship of *H. armigera* based on mitochondrial *COI gene*. Infested tomatoes with *H. armigera* larvae were collected from Çanakkale province (14 location), Balıkesir (1 location) and Bursa (1 location) in August and September 2017 and 2018. Larvae representing for each location (n=3) were used for genomic DNA isolation. The partial *COI* gene (536 bp) was amplified and PCR products were sequenced directly. The numbers of haplotypes and mutations were estimated using the DnaSP. Genetic relationships between the population were evaluated using the UPGMA method in MEGA X program. As a result, we identified 13 haplotypes and 13 mutations in all tested 45 samples. Additionally, 9 private haplotypes were detected among *H. armigera* population. Analyses revealed that population of *H. armigera* sampled from different geographic location were established as two distinct groups. The first group comprised 12 haplotypes (Çanakkale and Balıkesir population) while the distinct second group consisted of only 1 haplotype (Bursa population). The presented study was the first attempt to detect molecular characterization and genetic characterization of cotton bollworm in Turkey.
Keywords: *Helicoverpa armigera*, Cotton bollworm, Genetic diversity, mtDNA, *COI* gene.

Çanakkale'de Yeşil Kurt, *Helicoverpa armigera* (Lepidoptera: Noctuidae) Popülasyonunun Genetik Çeşitliliği

Öz

Yeşil Kurt, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) dünyada tarımsal alanlarda yaygın olarak bilinen polifag bir zararlıdır. Bu zararlı domates üretiminde kalite ve ürünlerde önemli zararlara sebep olup, Türkiye'de 1913 yılından beri bilinmektedir. Çalışmanın amacı, yeşil kurdun mitokondrial *COI* genindeki genetik çeşitliliği ve filogenetik benzerliğinin belirlenmesidir. 2017 ve 2018 yılları Ağustos ve Eylül aylarında yeşil kurt ile bulaşık domates örnekler Çanakkale (14 alandan), Balıkesir (1 alan) ve Bursa (1 alan) toplandı. Her örnekleme alanını temsil eden larvalar (n=3) kullanılarak genomik DNA izolasyonu yapıldı. *COI* geninin bir kısmı (536 bç) çoğaltılarak PCR ürünlerinin direkt olarak dizilimleri ortaya konuldu. DnaSP ile haploit sayıları ve mutasyonları ve populasyonlar arası genetik akrabalık düzeyleri UPGMA metodu ile MEGA X programında belirlendi. Sonuç olarak, test edilen 45 örnekte 13 haplotip ve 13 mutasyon tespit edildi. Buna ilave olarak yeşil kurtta 9 özel haplotip bulundu. Analizler sonucunda farklı coğrafik alanlardan elde edilen yeşil kurt popülasyonları 2 ayrı grupta yer aldı. İlk grup 12 haplotip (Çanakkale ve Balıkesir popülasyonu) ve diğer grupta ise sadece 1 haplotip (Bursa popülasyonu) yer aldı. Bu çalışma, Türkiye'de yeşil kurdun moleküler karakterizasyonu ve genetik çeşitliliğinin belirlenmesi amacıyla yapılan ilk çalışmadır.
Anahtar Kelimeler: *Helicoverpa armigera,* Yeşil kurt, Genetik çeşitlilik, mtDNA, *COI* gen.

Introduction

The cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), is a commonly known polyphagous pest in worldwide. This pest is distributed in agricultural areas in the Middle East, India, Africa, Eastern and Northern Australia, New Zealand, Southern Europe and Central and Southern Asia (Fitt, 1989). It was first recorded in 1913 in Turkey (Alkan, 1948). Immature stages of *Helicoverpa armigera* have been damaged to important crops such as cotton,

ÇOMÜ Zir. Fak. Derg. (COMU J. Agric. Fac.)
2022: 10 (1): 211–221
ISSN: 2147–8384 / e-ISSN: 2564–6826
doi: 10.33202/comuagri.1054611

tomato, maize, sorghum, tobacco, soybean, legumes, ornamental plants and fruit trees together with more than 67 host families (Pogue, 2004).

The damage is caused by larvae boring a hole as it feeds into the reproductive parts of the host. Larvae feeding on buds, developing pods, fruits and seeds, may cause to secondary infection by pathogens. The damage to tomato fruit production is severe, resulting in quantitative losses and reducing the quality of tomato. Although sex pheromone traps have been used to manage *H. armigera,* traditionally, the control of cotton bollworm is mostly based on the use of synthetic pyrethroid insecticides such as spinosad, thiodicarb, profenofos and pyridalyl in Turkey (Uğurlu and Gürkan, 2007).

The pest has high reproductive potential, laying an average of 1000 to 1500 eggs. Moreover, it is characterized by high mobility and fecundity for survival, high adaptability to various climatic conditions (Mironidis and Savopoulou-Soultani, 2008).

A better understanding about population genetic variation pattern and genetic differences of *H. armigera* is very important for understanding migration patterns, structure and population dynamics, local adaptation and their behavior, and may be very useful information for pest control (Asokan et al., 2012). Molecular marker technology has become an important tool in studies such as molecular identification and evolution. Especially, the mitochondrial genes have been commonly known as molecular tools for the history and evolution between closely related taxa, the analysis of population and evolutionary studies involving population genetics and phylogenetics (Simon et al., 1994; Behere et al., 2007a).

Mitochondrial DNA is maternally inherited and transmitted from generation to generation (Simon et al., 1994). An insect mitochondrial (mt) genome is about 14 to 18 kb in size. It is circular, consisting 37 genes; 13 of them are protein coding genes (PCGs), there are 22 tRNAs and 2 rRNAs genes and several noncoding regions (Boore, 1999). Sequences of mtDNA have been used for studies on population genetic studies of Lepidopteran pests, involving analyzes of population diversity, phylogenetic relationships and gene flow (Behere et al., 200b7; Albernaz et al., 2012). The cytochrome oxidase I (*COI*) region of mtDNA is the most extensively sequenced gene region of the insect mitochondrial genome.

The aim of study is to investigate genetic characterization and phylogenetic relationship of the cotton bollworm based on the mitochondrial COI gene in Çanakkale (Turkey).

Materials and Methods

Insect collections

Helicoverpa armigera individuals of different larvae stages were collected from Çanakkale (14 location), Balıkesir (1 location) and Bursa (1 location) during the 2017-2018 tomato production season (Figure 1 and Table 1).

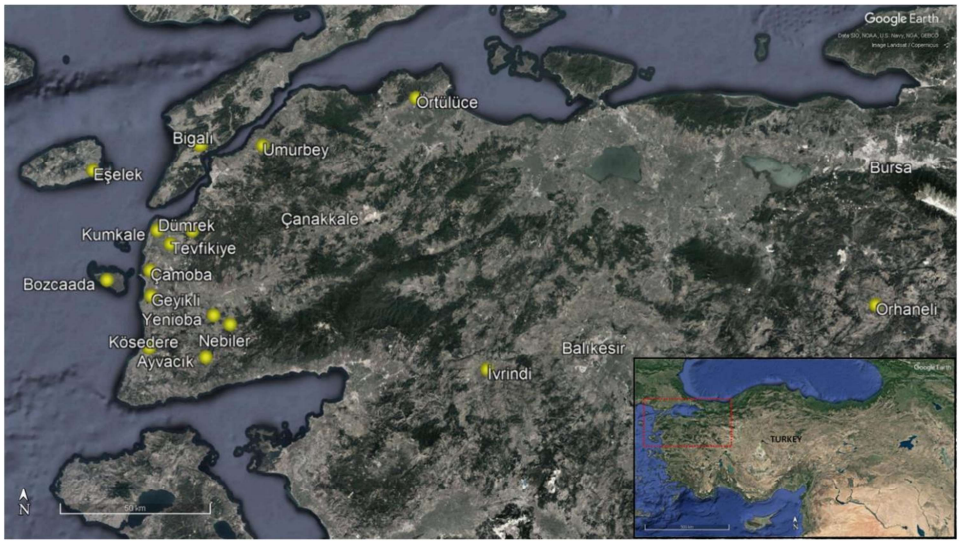


Figure 1. Collection sites of *H. armigera* from Çanakkale province, Turkey.

ÇOMÜ Zir. Fak. Derg. (COMU J. Agric. Fac.)
2022: 10 (1): 211–221
ISSN: 2147–8384 / e-ISSN: 2564–6826
doi: 10.33202/comuagri.1054611

Table 1. Collection sites of *H. armigera* specimens as larvae in Turkey.

Province/ Population	Sublocation	Population code	Crop	Collection Date	Specimens (N)
Çanakkale	Dümrek	DUM	Tomato	July, 2017	3
	Tevfikiye	TEV	Tomato	Aug., 2017	3
	Batakovası	BTK	Tomato	Aug., 2017	3
Ezine	Çamoba	CAM	Tomato	Aug., 2017	3
	Yenioba	YEN	Pepper	Aug., 2018	2
Ayvacık	Central	AYV	Tomato	Aug., 2018	3
	Kösedere	KOS	Tomato	Aug., 2017	3
Biga	Örtülüce	ORT	Tomato	Aug., 2018	3
Bayramiç	Nebiler	NEB	Tomato	Sept., 2017	3
Geyikli	Central	GYK	Tomato	Aug., 2017	3
Eceabat	Bigalı	BIG	Tomato	Aug., 2018	3
Bozcaada	Central	BOZ	Tomato	Aug., 2018	3
Lapseki	Umurbey	UMR	Tomato	Sept., 2018	3
Gökçeada	Eşelek	ESE	Tomato	Sept., 2018	3
Bursa	Orhaneli	BRS	Tomato	Sept., 2017	2
Balıkesir	İvrindi	BAL	Tomato	Sept., 2018	2

Collections were made from 15 population on tomato (*Solanum lycopersicum*, Solanaceae) and one population of pepper (*Capsicum annuum*, Solanaceae). Different stages of larvae (first to sixth instar) were placed individually in an eppendorf tube. A total of 45 samples were analysed for this study. Samples from each location were preserved in 100% ethanol and stored at -20˚C before DNA extraction.

DNA extraction, PCR amplification and sequencing

The genomic DNA was isolated from the posterior portion of individual larvae (Behere et al., 2013) using a GeneJET Genomic DNA Extraction Kit (Thermo Scientific™, Cat. K0721), followed by the manufacturer's protocol. DNA concentration was estimated with a NanoDrop (Thermo Scientific™, One/OneC Microvolume). The COI mitochondrial gene was used for genetic analysis. The partial sequences of the COI gene were amplified by PCR using the universal primers LCO1490 (F) and HCO2198 (R) (Folmer et al., 1994).

The PCR reactions were performed by adding one µl of DNA (20-30 ng/µL), 1 µL $MgCl_2$ (25 mM), 0.5 µL each of primers (10 pmol), 0.5 µL dNTP's (10 mM), 2.5 µL PCR reaction buffer (Ampliqon), 0.1 µL Taq DNA polymerase (Ampliqon) and 18.9 µL PCR Grade water (Ampliqon) in a final volume of 25 µL. Reactions were performed on a Bio-Rad S1000TM Thermal cycler. The PCR program had a denaturation at 94˚C for 4 min, followed by 35 cycles of denaturation at 94˚C for 40 s, annealing at 48˚C for 1 min, and extension at 72˚C for 45 s, with a final extension step at 72˚C for 10 min. After amplification, 3 µL of the PCR reaction were analyzed by 1% (w/v) agarose gel electrophoresis in 1X TAE at 120V for 30 min and stained with 3 µL ethidium bromide and visualized on a UVP Transilluminator.

Data and phylogenetic analyses

All sequences were manually assembled, edited and trimmed using MEGA X software program. Then, Clustal W (1.6) were used to align sequences (Thompson et al., 1994). The mtDNA sequences were analyzed with neighbor-joining (NJ) clustering method (Saitou and Nei, 1987). A dendogram representing the phenetic relationships among *H. armigera* samples was obtained by MEGA X software (Kumar et al., 2018). The confidence of each branch was found by using the non-parametric bootstrapping with 1000 replicates (Felsenstein, 1985).

ÇOMÜ Zir. Fak. Derg. (COMU J. Agric. Fac.)
2022: 10 (1): 211–221
ISSN: 2147–8384 / e-ISSN: 2564–6826
doi: 10.33202/comuagri.1054611

Genetic diversity analyses were estimated with DnaSP v6 (Rozas et al., 2017) and Arlequin v.3.5 (Excoffier and Lischer, 2010). Estimates of haplotype diversity ($h \pm$ SE) and nucleotide diversity ($\pi \pm$ SE) parameters, as defined by Nei (1987), were estimated using Arlequin v.3.5. The p-distance model were used for genetic diversity of haplotypes. Additionally, the numbers of haplotypes and mutations, the numbers of polymorphic sites and pairwise nucleotide differences, the parsimony informative sites and singleton sites were identified.

The demographic status of the tested population were characterized by the segregation of paired differences among sequences as implemented in DnaSP v.6 (Rozas et al., 2017). The recently expended population have described by unimodal distribution, whereas samples drawn from population at demographic equilibrium present a multimodal distribution (Rogers and Harpending, 1992) and the demographic expansion Tau (τ) parameter (α=0.05 and 1000 permutations). In order to test for population expansion we also implemented two statistical tests often used to analyze demographic events. Besides, selective neutrality tests based on Tajima's parameter *D* (Tajima, 1989) and Fu's parameter F_s (Fu, 1997) were estimated. Statistical significance was assessed using 1000 permutations.

The mtDNA generated has been deposited in GenBank (Accession numbers: MN132887-MN132931). We also compared our COI data to the available data in GenBank. Phylogenetic analysis was performed with the unweighted pairgroup method with arithmetic averages (UPGMA) method (1000 bootstrap replicates) with Kimura two-parameters (K2P; Kimura 1980) method in the MEGA X (Kumar et al., 2018). Here, *Spodoptera frugiperda* (GU439148) served as outgroup. Apart from that, to display the diversity of haplotypes, we used the program PopART v1.7 (Leigh and Bryant, 2015) to construct the Median Joining Network (MJ NETWORK).

Results

A total of 45 samples of *H. armigera* amplified a 613-bp fragments of COI gene and was successfully sequenced (Figure 2).

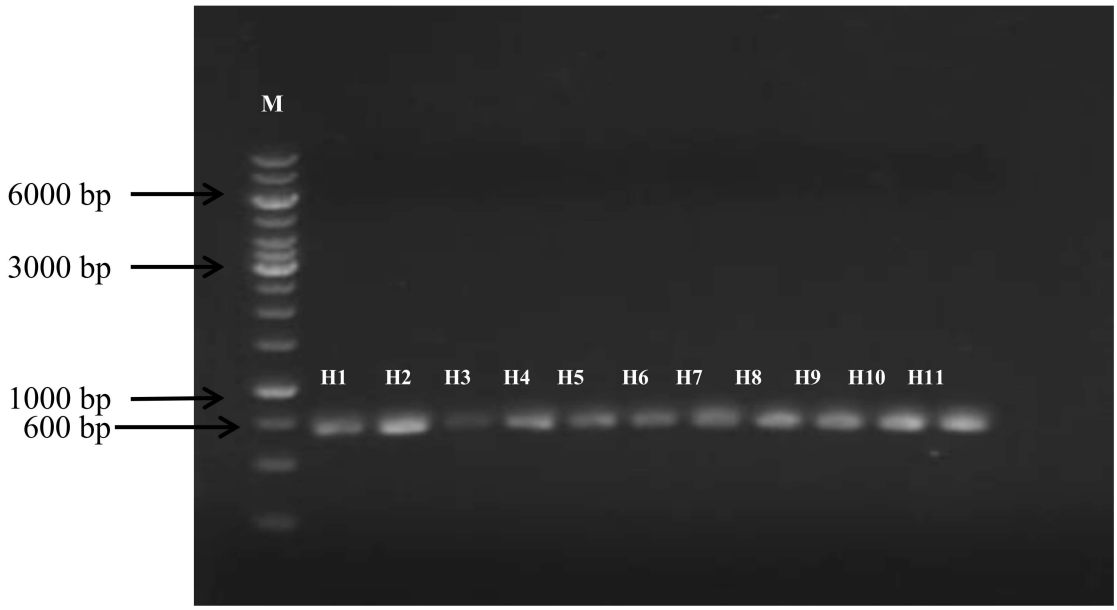


Figure 2. Amplified COI fragments (536 bp) of *H. armigera* from different locations with bands seperated on 1% agarose gel (M: Molecular marker; 10000 bp DNA ladder, Fermentas).

In order to eliminate the errors due to sequencing artifacts, we trimmed the beginning and end of the sequences. After editing, aligning and trimmed the sequences, we characterized the 536 bp consensus sequence. The COI sequences obtained for the samples from 16 localities were blasted and checked in GenBank database. Finally, all 45 samples matched 99-100% with *H. armigera* sequences. The 536 bp COI gene of *H. armigera* sampled from 16 localities revealed 13 haplotypes. Haplotype H5 was the most shared haplotype found in 11 populations/localities. Haplotype H1 was the second

ÇOMÜ Zir. Fak. Derg. (COMU J. Agric. Fac.)
2022: 10 (1): 211–221
ISSN: 2147–8384 / e-ISSN: 2564–6826
doi: 10.33202/comuagri.1054611

most shared haplotype found in 10 populations/localities. Haplotype H3 was shared by 5 locations. One of the COI gene haplotypes (H12) was only shared by populations from Bursa (Table 2). No insertions or deletions were observed in the sequences.

Table 2. Distribution of *H. armigera* mitochondrial haplotypes shared by different locations.

COI Haplotype	Number of individuals from population of each location																
	DUM (3)	TEV (3)	BTK (3)	CAM (3)	YEN (2)	AYV (3)	KOS (3)	ORT (3)	NEB (3)	GYK (3)	BIG (3)	BOZ (3)	UMR (3)	ESE (3)	BRS (2)	BAL (2)	Total (45)
H1	2		1	2	2		1		1		2		1	1		1	14
H2	1																1
H3		1				1		1				1		1			5
H4		1	1														2
H5		1		1		1	2	2	2	1	1	1	1	1			14
H6			1														1
H7						1											1
H8										1							1
H9										1							1
H10												1					1
H11													1				1
H12															2		2
H13																1	1

The number of haplotypes and mutations are shown in Table 3. We identified 13 haplotypes and 13 mutations. In addition to that, 9 private haplotypes were detected among *H. armigera* tested population. Sequences contained 523 conserved sites and 13 variable sites, 6 of which were singletons (at sites 186, 195, 304, 310, 384 and 534) and 7 were parsimony informative (at sites 138, 173, 218, 226, 231, 303 and 414). Eight substitutions occured at the third position (61.5%), two replacements were determined at a second codon position (15.4%), and four replacements were at first codon positions (23.1%). Of the translated 178 amino acids, there were 4 substitutions without any non-sense amino acids. The average base frequences were A:30.70%, T:40.30%, C:15.86% and G:13.14%, indicating a strong AT bias (71%) which is typical of mitochondrial genes and not observed mitochondrial DNA-like sequences in the nucleus (numts) (Zhang and Hewitt, 1996). The transition/transversion (Ts/Tv) ratios were k_1= 111.26 (purines) and k_2= 51.401 (pyrimidines). There was only one transversion (at site 310) and 12 transitions (in all other substitutions sites) with the most frequently observed transition being C/T (49.4) followed by T/C (19.73). The overall was calculated as R = 31.574 (Tamura et al., 2004). The mean number of nucleotide differences was k=1.448. The value of τ = 1.000. The mismatch distribution demonstrated a unimodal motif of the *H. armigera* haplotypes (Figure 3). The demographic distribution was carried on by the neutrality tests: Fu's F_s = -7.423 ($P < 0.001$) and Tajima's D = -1.56598 ($P > 0.10$).

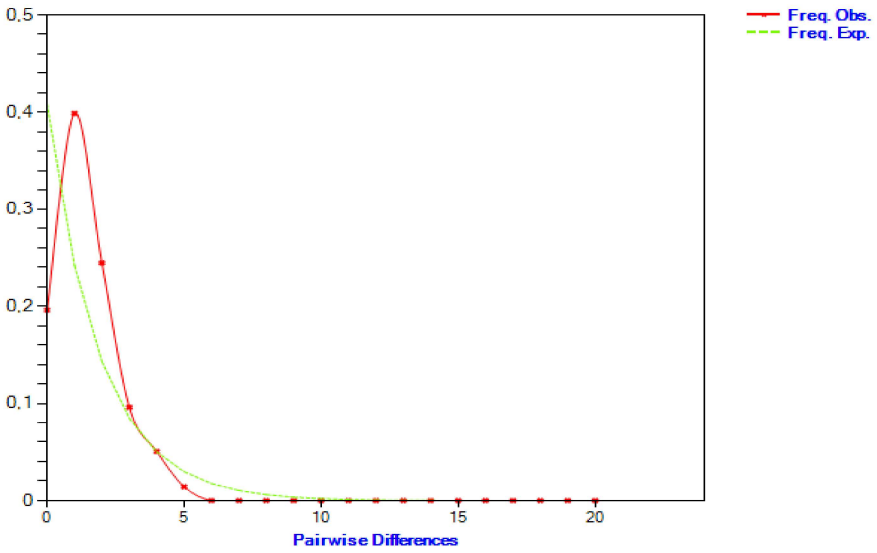


Figure 3. Mismatch distributions for collected *H. armigera* populations based on mtDNA COI gene.

ÇOMÜ Zir. Fak. Derg. (COMU J. Agric. Fac.)
2022: 10 (1): 211–221
ISSN: 2147–8384 / e-ISSN: 2564–6826
doi: 10.33202/comuagri.1054611

In general, high haplotype diversity (average-*h* =0.804%) and high nucleotide diversity (average-π=0.270%) were observed in the sampled locations (Table 3). Haplotype diversity and nucleotide diversity ranged from 1.0±0.5 to 0.00 and 0.37±0.13 to 0.00, respectively. The highest haplotype diversity observed in Ivrindi location (*h* =1.00±0.5) while the lowest haplotype diversity observed in Yenioba and Orhaneli location (*h* =0.00). Second highest haplotype diversity observed in Tevfikiye, Batakovası, Ayvacık, Geyikli, Bozcaada, Umurbey and Eşelek locations. On the other hand, in Yenioba and Orhaneli locations were shown the lowest diversity indices (π=0.00), while that in Batakovası, Ayvacık, Geyikli and Bozcaada locations had the highest diversity values (π=0.37±0.13).

Table 3. Comparison of *H. armigera* mtDNA partial COI haplotype diversity (*h*) and nucleotide diversity (π) between different locations[1]

Population	Location	N	COI gene				
			S	N_h	Unique N_h	H_d	π (%)
Çanakkale	Dümrek	3	1	2	1	0.667±0.314	0.124±0.059
	Tevfikiye	3	2	3	-	1.000±0.272	0.249±0.083
	Batakovası	3	3	3	1	1.000±0.272	0.373±0.131
	Çamoba	3	1	2	-	0.667±0.314	0.124±0.059
	Yenioba	2	0	1	-	0.000±0.000	0.000±0.000
	Ayvacık	3	3	1	1	1.000±0.272	0.373±0.131
	Kösedere	3	1	2	-	0.667±0.314	0.124±0.059
	Örtülüce	3	1	2	-	0.667±0.314	0.124±0.059
	Nebiler	3	1	2	-	0.667±0.314	0.124±0.059
	Geyikli	3	3	3	2	1.000±0.272	0.373±0.131
	Bigalı	3	1	2	-	0.667±0.314	0.124±0.059
	Bozcaada	3	3	1	1	1.000±0.272	0.373±0.131
	Umurbey	3	2	3	1	1.000±0.272	0.249±0.083
	Eşelek	3	2	3	-	1.000±0.272	0.249±0.083
Bursa	Orhaneli	2	0	1	1	0.000±0.000	0.000±0.000
Balıkesir	İvrindi	2	1	2	1	1.000±0.500	0.187±0.093
Average	-	45	13	13	9	0.804±0.040	0.270±0.037

[1] 536 bp of COI gene sequences; N: number of sequences of each population; S: number of segregating sites; N_h: number of haplotypes; H_d: haplotype diversity; π: nucleotide diversity.

Table 4 showed pairwise matrix among *H. armigera* haplotypes. The haplotypes ranged from 0.18 % to 0.93 % (1 to 5 bp). The haplotype H12 from Bursa had the highest nucleotide difference as 5 bp (Table 4).

Table 4. Pairwise comparisons among *H. armigera* haplotypes. Percent differences are indicated in the lower part of the diagonal, and the net number of nucleotide differences is shown in the upper part of the diagonal.

	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13
H1		1	2	2	1	1	3	1	2	1	1	4	1
H2	0.002		3	3	2	2	4	2	3	2	2	5	2
H3	0.004	0.006		2	1	3	3	3	2	3	3	4	3
H4	0.004	0.006	0.004		1	3	1	3	2	3	3	4	3
H5	0.002	0.004	0.002	0.002		2	2	2	1	2	2	3	2
H6	0.002	0.004	0.006	0.006	0.004		4	2	3	2	2	5	2
H7	0.006	0.007	0.006	0.002	0.004	0.007		4	3	4	4	5	4
H8	0.002	0.004	0.006	0.006	0.004	0.004	0.007		3	2	2	5	2
H9	0.004	0.006	0.004	0.004	0.002	0.006	0.006	0.006		1	3	4	3
H10	0.002	0.004	0.006	0.006	0.004	0.004	0.007	0.004	0.002		2	5	2
H11	0.002	0.004	0.006	0.006	0.004	0.004	0.007	0.004	0.006	0.004		5	2
H12	0.007	0.009	0.007	0.007	0.006	0.009	0.009	0.009	0.007	0.009	0.009		5
H13	0.002	0.004	0.006	0.006	0.004	0.004	0.007	0.004	0.006	0.004	0.004	0.009	

The pairwise comparisons of the fixation index, F_{st} were utilized to conduct the genetic differences among *H. armigera* haplotypes. Pairwise F_{st} values among haplotypes varied from 0 to 1. Bursa population differs from Çamoba, Dümrek, Bigalı, Geyikli, Eşelek, Kösedere, Batakovası,

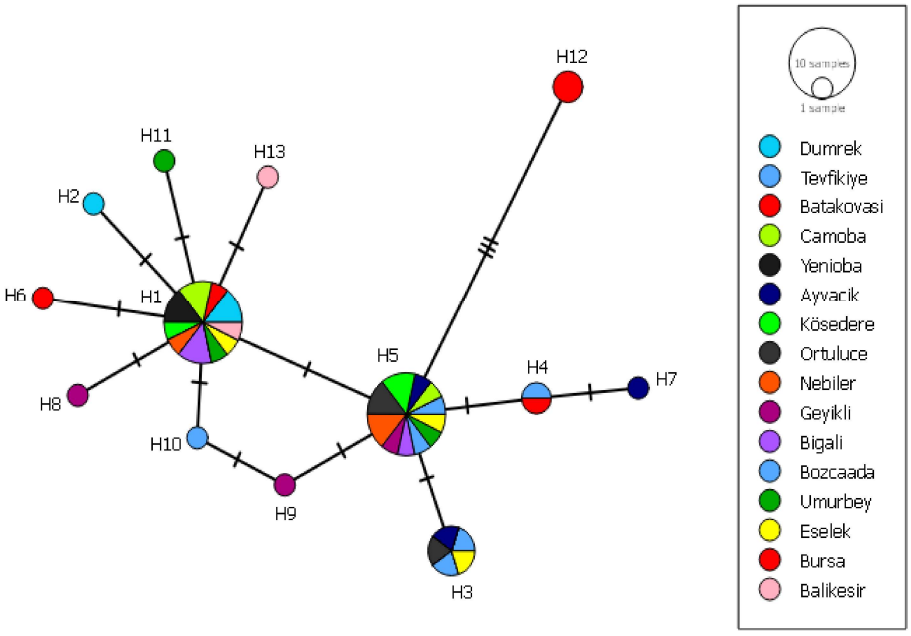
ÇOMÜ Zir. Fak. Derg. (COMU J. Agric. Fac.)
2022: 10 (1): 211–221
ISSN: 2147–8384 / e-ISSN: 2564–6826
doi: 10.33202/comuagri.1054611

Nebiler, Tevfikiye and Umurbey populations (Fst > 0.75). Balıkesir population and Bursa population showed genetic differentiation values on the scale of 0.89. The F_{st} value was highest among Bursa population and Yenioba population (Fst=1). The data also show, even subtly, that Bursa population showed some differences from others (Table 5).

Table 5. F_{st} values to compare among *H. armigera* populations.

	AYV	BAL	ORT	BOZ	BRS	CAM	DUM	BIG	GYK	ESE	KOS	BTK	NEB	TEV	UMR
AYV															
BAL	0.40														
ORT	0	0.55													
BOZ	0	0.18	0												
BRS	0.75	0.89	0.9	0.75											
CAM	0.20	0	0.33	0	0.91										
DUM	0.43	0	0.6	0.2	0.92	0									
BIG	0.2	0	0.33	0	0.91	0	0								
GYK	0	0.18	0	0	0.75	0	0.2	0							
ESE	0	0.22	0	0	0.82	0	0.25	0	0						
KOS	0	0.29	0	0	0.9	0	0.33	0	0	0					
BTK	0.05	0	0.2	0	0.77	0	0	0	0	0	0				
NEB	0	0.29	0	0	0.9	0	0.33	0	0	0	0	0			
TEV	0	0.46	0	0	0.82	0.25	0.5	0.25	0	0	0	0.06	0		
UMR	0.17	0	0.25	0	0.83	0	0	0	0	0	0	0	0	0.2	
YEN	0.5	0	0.75	0.25	1	0	0	0	0.25	0.33	0.5	0	0.5	0.6	0

The Median-Joining network method demostrated that the two common haplotypes (H1 and H5) holding in the middle position. Haplotype H5 occur in eleven locations (Tevfikiye, Çamoba, Ayvacık, Kösedere, Örtülüce, Nebiler, Geyikli, Bigalı, Bozcaada, Umurbey and Eşelek) while haplotype H1 occur in ten locations (Dümrek, Batakovası, Çamoba, Yenioba, Kösedere, Nebiler, Bigalı, Umurbey, Eşelek and Balıkesir). Haplotype H5 and H1, representing approximately 63% (28/45) of the total sample. Haplotype H3 is restricted to five locations (from Tevfikiye, Ayvacık, Örtülüce, Bozcaada and Eşelek) and haplotype H4 to two locations (Tevfikiye and Batakovası). The haplotype H3 represented nearly 12% of the total samples. Most of the other *H. armigera* haplotypes (9 haplotypes; H2, H6, H7, H8, H9, H10, H11, H12 and H13) were from single location (Figure 4).



ÇOMÜ Zir. Fak. Derg. (COMU J. Agric. Fac.)
2022: 10 (1): 211–221
ISSN: 2147–8384 / e-ISSN: 2564–6826
doi: 10.33202/comuagri.1054611

Figure 4. Median-Joining Network based on partial mtDNA COI (536 bp) of *H. armigera* samples. Color coded locations are shown in the squares. Each haplotype is represented by a circle and identified by a number H1-H13. The area of a circle is proportional to the number of observed individuals.

Neighbor- joining (NJ) distance analysis (Saitou and Nei, 1987) and sequence distribution with Kimura two-parameters (K2P; Kimura, 1980) models guided for the COI sequences demonstrated almost the same tree topologies. Taken as a whole, 2 individuals from Bursa population and 8 individuals from Çanakkale and Balıkesir population, while the other 35 individuals seperated by a knot with 96% of consistency (Figure 5).

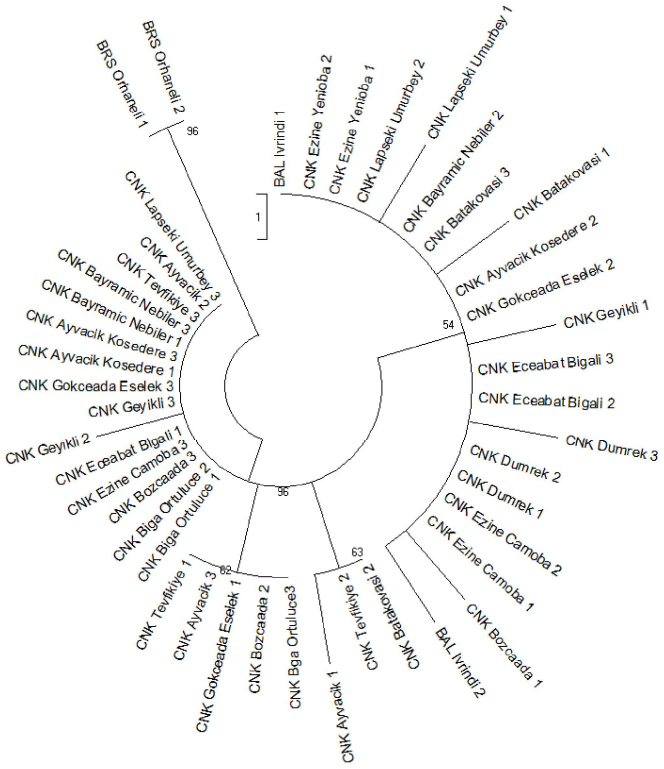


Figure 5. Relationships among individuals of *H. armigera* samples from the COI gene (536 bp). Numbers on branches indicate values of 1000 bootstrap replicates. Codes represent sampling localities of *H. armigera* population, as detailed in Table 1.

The phylogenetic tree was constructed using COI nucleotide sequences of *H. armigera* population. The unweighted pair group method with arithmetic mean (UPGMA) method tree revealed that there were two distinct groups, in which the first group comprised twelve population while the distinct second group consisted of only Bursa population. The first group was subdivided into A and B whereas second group only one population from Bursa. The A group comprising 8 haplotypes (H1, H2, H6, H8, H9, H10, H11 and H13; respectively from Dümrek, Batakovası, Çamoba, Yenioba, Kösedere, Nebiler, Bigalı, Umurbey, Eşelek, Geyikli, Bozcaada in Çanakkale and in Balıkesir) and B group comprising 4 haplotypes (H3, H4, H5 and H7; respectively from Tevfikiye, Ayvacık, Örtülüce, Bozcaada, Eşelek, Çamoba, Kösedere, Nebiler, Geyikli, Bigalı, Umurbey, Eşelek, Batakovası in Çanakkale). The UPGMA tree showed that there was a very close genetic similarity among population of *H. armigera* in Çanakkale and Balıkesir population. Geyikli and Bozcaada population showed some differences from others. Bursa population was genetically very different (Figure 6).

ÇOMÜ Zir. Fak. Derg. (COMU J. Agric. Fac.)
2022: 10 (1): 211–221
ISSN: 2147–8384 / e-ISSN: 2564–6826
doi: 10.33202/comuagri.1054611

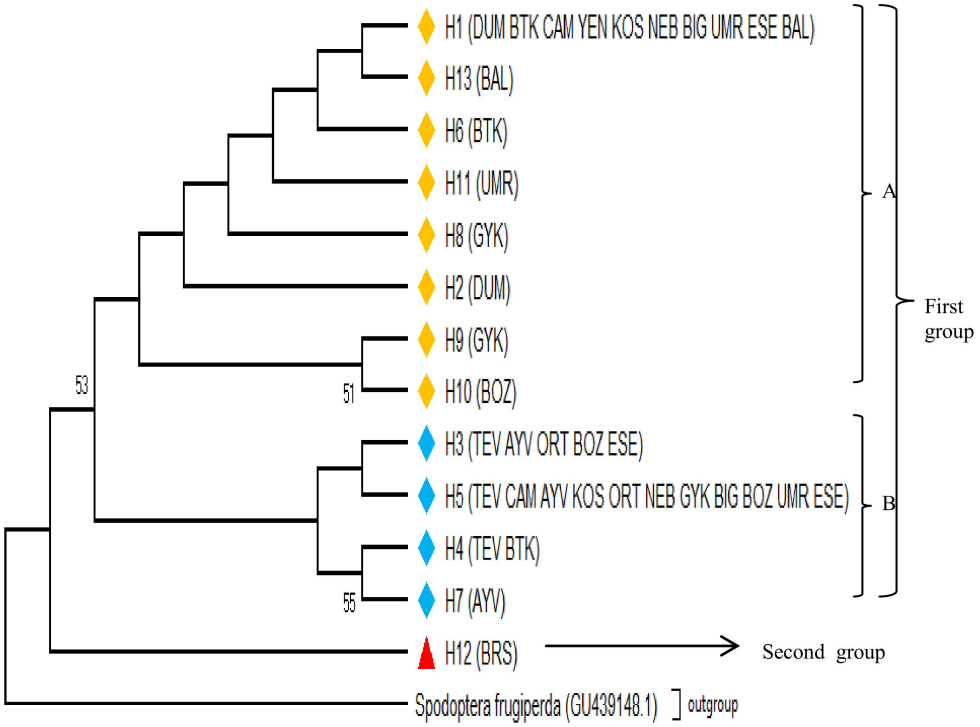


Figure 6. Unweighted pair group method with arithmetic mean (UPGMA) phenogram of the association of *H. armigera* samples. *Spodoptera frugiperda* was used as outgroup.

Discussion

Mitochondrial DNA markers such as COI, COII, microsatellites, RAPDs, AFLP analysis, ribosomal RNA have been commonly used in evolutionary and population structure studies in many countries (Behere et al., 2013; Perera et al., 2015). Eventhough the widely distribution of *H. armigera* in Turkey, genetic chracterization and diversity were not known in Turkey.

Presented work on the genetic diversity of *H. armigera* from Çanakkale province assessed by COI region of mitochondrial DNA resulted haplotype with nucleotide diversity as 0.804 and 0.270 in collected sample. High genetic diversity has been reported for *H. armigera* in different regions (Albernaz et al., 2012; Leite et al., 2014). Genetic diversity estimates indicated high molecular variation.

The studies described here indicate the first and recent demographic expansion for *H. armigera* in Çanakkale province, Turkey. This demographic expansion was also supported by the unimodal distribution of paired differences between sequences and the significant negative values of neutrality tests for the Tajima's *D* and Fu's F_s parameters. Besides, population growth also relies on pairwise divergence among sequences and the number of segregating sites among the sequences (Albernaz et al., 2012). We conclude that negative values of neutrality tests shows that there are unique alleles in the populations and a low rate of heterozygousity (Excoffier et al., 2009).

In conclusion, we analyzed both Median-Joining network analysis and UPGMA phenogram of *H. armigera* populations sampled from different places. According to the MJ network tree and UPGMA tree, there was a very close genetic similarity among population of *H. armigera* in Çanakkale and Balıkesir population. Also, it was determined that Bursa population was genetically quite different and distant from other populations.

Conclusion

The study reports the first molecular evidence and genetic diversity on the occurrence of *H. armigera* in Turkey. Genetic analyses based on the use mitochondrial genes can provide useful tools

for genetic and phylogenetic relationships in *H. armigera* population in Turkey. The phylogenetic relationship of *H. armigera* population is important to understand pest population dynamics, population structure and gene flow. Therefore, the presented data will be crutical for developing strategies to conduct the further evolution of insect resistance to insecticides and integrated pest control programmes.

Acknowledgement: This research was financially supported by Canakkale Onsekiz Mart University, Scientific Research Projects Commission (FYL-2018/2466). The authors thank Emeritus Prof. Dr. James L. Nation for reviewing and his useful suggestions. The part of this study was published as an abstract in the 1st International Molecular Plant Protection Congress, April 10-13, 2019 in Adana, Turkey.

Authors' Contributions

The contribution of the authors is equal.

Conflicts of Interest Statement

Authors have declared no conflict of interest.

References

Albernaz, K.C., Silva-Brandao, K.L., Fresia, P., Consoli, F.L., Omoto, C., 2012. Genetic variability and demographic history of *Heliothis virescens* (Lepidoptera: Noctuidae) populations from Brazil inferred by mtDNA sequences. Bulletin of Entomological Research. 102: 333-343.

Alkan, B., 1948. Orta Anadolu Hububat Zararlıları (Zararlı Hayvan ve Böcekler). A.Ü. Ziraat Fakültesi Yayınları. Sayı:1, Ankara Üniversitesi Basımevi, Ankara, 71 s.

Asokan, R., Rebijith, K.B., Krishna Kumar, N.K., Manamohan, M., 2012. Genetic diversity of tomato fruit borer, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) inferred from mitochondrial cytochrome oxidase-I (mtCO-I). Pest Management in Horticultural Ecosystems. 18 (1): 29-34.

Behere, G.T., Tay, W.T., Russell, D.A., Heckel, D.G., Appleton, B.R., Kranthi, K.R., Batterham, P., 2007a. Mitochondrial DNA analysis of field populations of *Helicoverpa armigera* (Lepidoptera: Noctuidae) and of its relationship to *H. zea*. BMC Evolutionary Biology. 7: 117-127.

Behere, G.T., Tay, W.T., Russell, D.A., Batterham, P., 2007b. Molecular markers to discriminate among four pest species of *Helicoverpa* (Lepidoptera: Noctuidae). Bulletin of Entomological Research. 98: 599-603.

Behere, G.T., Tay, W.T., Russell, D.A., Kranthi, K.R., Batterham, P., 2013. Population genetic structure of the cotton bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in India as Inferred from EPIC-PCR DNA Markers. PLoS ONE. 8: e53448.

Boore, J.L. 1999. Animal mitochondrial genome. Nucleic Acids Research. 27: 1767-1780.

Excoffier, L., Foll, M., Petit, R.J., 2009. Genetic consequences of range expansions. Annual Review of Ecology Evolution and Systematics. 40: 481-501.

Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources. 10: 564-567.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783-791.

Fitt, G.P., 1989. The ecology of *Heliothis* species in relation to agroecosystems. Annual Review of Entomology. 34: 17-52.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology. 3: 294-299.

Fu, Y.X., 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics. 147:915-925.

Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution. 16: 111-120.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution. 35: 1547-1549.

Leigh, J.W., Bryant, D., 2015. PopART: Full-feature software for haplotype network construction. Methods in Ecology and Evolution. 6: 1110-1116.

Leite, N.A., Alves-Pereira, A., Corrêa, A.S., Zucchi, M.I., Omoto, C., 2014. Demographics and genetic variability of the New World Bollworm (*Helicoverpa zea*) and the Old World Bollworm (*Helicoverpa armigera*) in Brazil. PLoS One .9:e11328.

Mironidis, G.K., Savopoulou-Soultani, M., 2008. Development, survivorship and reproduction of *Helicoverpa armigera* (Lepidoptera: Noctuidae) under constant and alternating temperatures. Environmental Entomology. 37: 16-28.

Nei, M., 1987. Molecular evolutionary genetics. Columbia University Press, New York, NY. 512 pp.

Perera, O.P., Allen, K.C., Jain, D., Purcell, M., Little, N.S., Luttrell, R.G., 2015. Rapid Identification of *Helicoverpa armigera* and *Helicoverpa zea* (Lepidoptera: Noctuidae) Using Ribosomal RNA Internal Transcribed Spacer 1. Journal of Insect Science. 15 (1): 1-10.

Pogue, M.G., 2004. A new synonym of *Helicoverpa zea* (Boddie) and differentiation of adult males of *H. zea* and *H. armigera* (Hübner) (Lepidoptera: Noctuidae: Heliothinae). Annals of the Entomological Society of America. 97: 1222-1226.

Rogers, A.R., Harpending, H., 1992. Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution. 9: 552-569.

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E., Sánchez-Gracia, A., 2017. DNA sequence polymorphism analysis of large data sets. Molecular Biology and Evolution 34. (12): 3299-3302.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution. 4: 406-425.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting, and a phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction. Annals of the Entomological Society of America. 87: 651-701.

Tajima, F., 1989. The effect of change in population size on DNA polymorphism. Genetics. 123: 585-595.

Tamura, K., Nei, M., Kumar, S., 2004. Prospects for inferring very large phylogenesis by using the neighbor-joining method. Proceedings of the National Academy of Sciences of the USA. 101: 11030-11035.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research. 22 (22): 4673-4680.

Uğurlu, S., Gürkan, M.O., 2007. Insecticide resistance in *Helicoverpa armigera* from cotton-growing areas in Turkey. Phytoparasitica. 35 (4):376-279.

Zhang, D.X., Hewitt, G.M., 1996. Nuclear integrations: challenges for mitochondrial DNA markers. Trends in Ecology & Evolution. 11: 247-251.