Genetic Variation of *Chilo suppressalis* Walker (Lepidoptera: Pyralidae) Populations in Guilan and West of Mazandaran Provinces analysed with RAPD Markers

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Abstract

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Genetic variation of striped stem borer *Chilo suppressalis* populations in Guilan and Mazandaran provinces in Iran was studied in 2010 by means of RAPD markers. Collected 45 samples from 19 locations (representing 17 counties in Guilan province and 2 counties in Mazandaran province) were classified into four groups of populations: three population groups corresponding to the west, centre, and east of Guilan and one population group from the west of Mazandaran. RAPD PCR analysis showed that *Chilo suppressalis* populations in Mazandaran are genetically different from the Guilan populations while the western populations of Guilan are genetically separated from the two other populations. The central and the eastern populations of Guilan exhibit some similarities with those of Mazandaran and the similarities are more obvious between the eastern populations of Guilan and the western population of Mazandaran. The population from the centre of Guilan showed the highest level of intra-population genetic variation possibly due to wider rice cultivated area and/or due to higher sample size. The results showed a high level of genetic variation in *Chilo suppressalis* populations in the two provinces in Iran. In spite of the model species is a widely distributed pest, the study indicated that the samples originated in populations with a different genetic make-up.

Keywords: RAPD PCR; rice cultivate; Rice striped stem borer

Rice striped stem borer (RSSB) (*Chilo suppressalis* Walker: Pyralidae, Crambinae) is a major and destructive pest in rice fields in Guilan and Mazandaran provinces in Iran. Larvae bore into the stem and feed on the inner surface of the stem walls, which makes severe damage on rice plants. RSSB is a widespread species, extending from Asia and Oceania into the Middle East and Europe (Khan *et al*. 1991). It is regarded one of the most important rice pests in East Asia, India, and Indonesia. This pest was introduced to Iran in 1972 and has been widely distributed in all rice cultivated areas of Iran (Ebert 1972). Its distribution is random or aggregative in the field and it has 2–3 generations per year. In the northern parts of Iran, this pest has been distributed in all areas where its population density is usually higher than economic injury level. Its occurrence has been reported from other provinces of Iran such as Isfahan, Shiraz, Eilam, and Khozestan in 1995 where it caused severe damages (Moghaddas & Nasiri 1995). RSSB as a predominant and widely distributed pest is responsible for a large amount of rice crop losses despite the use of insecticides and the introduction of high-yielding varieties. Therefore, the development and use of rice varieties tolerant to RSSB and also pheromone traps for monitoring this pest have received increasing attention in Iran in recent years (Saeb *et al*. 2002). For breeding a rice plant resistant

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to a pest like RSSB it is necessary to identify and characterise the most useful genes and gene combinations in the host germplasm. It is also important to know the genetic structure of the pest population in order to predict the interactions between for identifying and characterising the resistance, because when a resistance gene is deployed in field and, it may face different populations of the pest, therefore it is important to characterise each resistance gene in terms of its spectrum of resistance relative to the pest populations. In addition, study about the genetic structure of a pest is an important aspect for understanding speciation, adaptation or genetic changes in organisms' populations (Syamsuardi & Okada 2002). For instance, resistant species are those species that express the genetic variation required to evolve mechanisms to escape control, these species are a symptom of the intensive selection pressure applied over wide area (Stanklewicz *et al*. 2001). So far, morphological characteristics, protein and metabolite profiles have been routinely used to study the population structure of pests (Loxdale *et al*. 1996). Molecular data based on the analysis of DNA polymorphisms have been used more recently to describe the genetic variation and population structure of various pests (e.g. Lima *et al*. 2002). These studies can serve as a basis for designing breeding strategies for any specific plants in any particular geographical regions or in pest resistance management. Up to now, despite the significance of RSSB, no assessment of the study about the genetic structure of RSSB by using molecular markers has been done in Iran. Papers on RSSB in other countries are limited study on geographic variation in susceptibility to *Bacillus thuringiensis* in China (Meng *et al*. 2003), effects of Bt transgenic rice on RSSB and its consequences on egg parasitoid, *Trichogramma brassicae* (Marzban 2012), RSSB resistance to pesticides (Cao *et al*. 2004; Li *et al*. 2007), biochemical study (Zibaii 2008), geometric morphometric study (Zahiri *et al*. 2006), isolation and characterisation of four polymorphic microsatellite loci (Jiang *et al*. 2005; Ishiguro *et al*. 2006; Meng *et al*. 2008), characterisation of complete mitochondrial genomes (Chai *et al.* 2012), and limited divergence among RSSB populations caused by gene flow in southeast China (Yang *et al*. 2012). The objective of the present first study on genetic diversity of *Chilo suppressalis* in Iran is to explore the level of RAPD genetic variation among active RSSB populations in Guilan and west part of Mazandaran provinces, without any distinctive morphological deferens.

MATERIAL AND METHODS

Sampling. 4th or 5th instar larvae of *Ch. supperssalis* were collected from harvested rice straw between

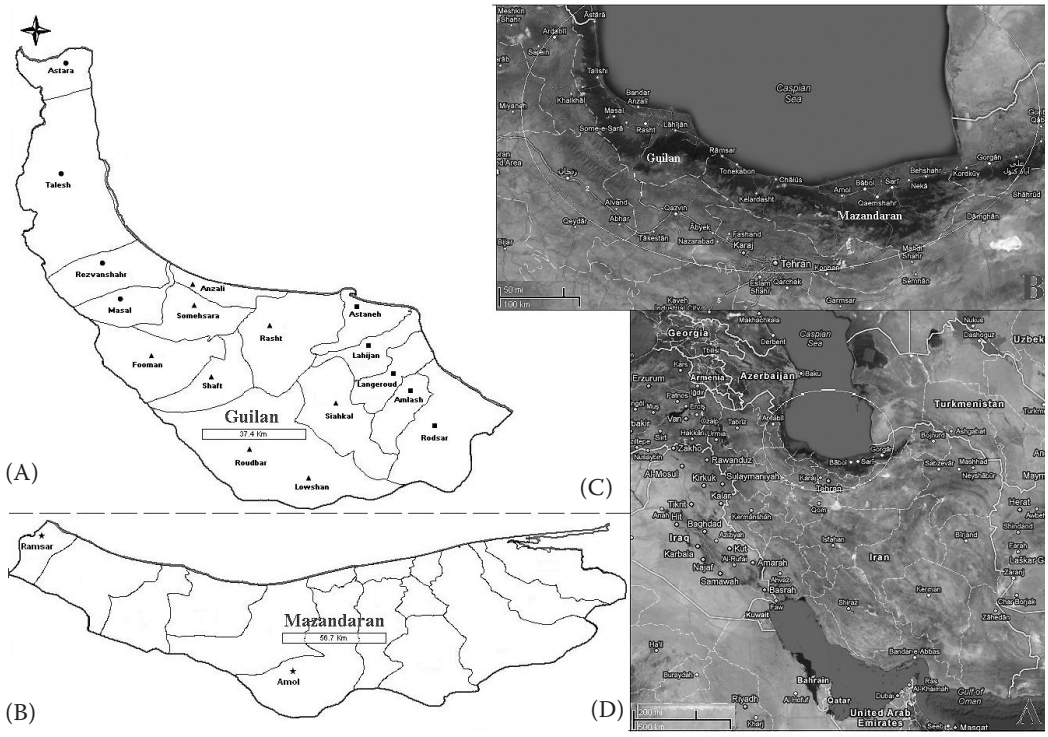


Figure 1. Overall map of the investigated area: (A) Inan map; (B) Northern provinces of Iran; (C) Guilan sampling areas; (D) Mazandaran sampling areas

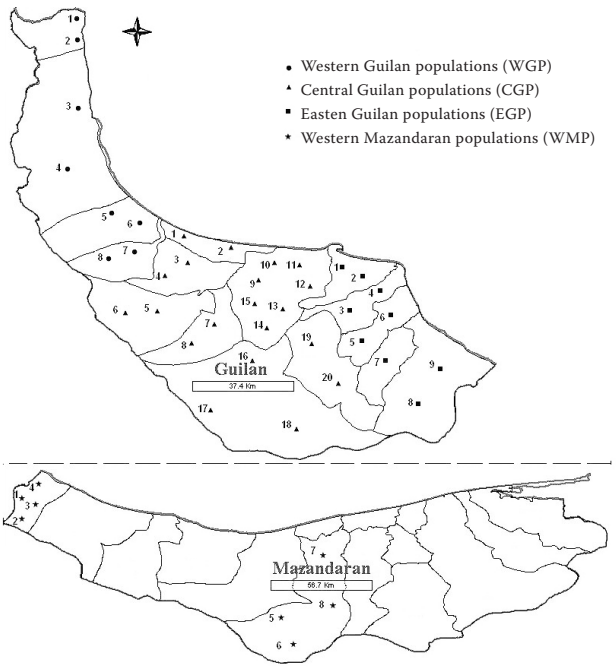


Figure 2. Geographical distribution of studied individuals on the Iranian territory

August and October 2009 and stored at –20°C for the subsequent molecular assays. In total, 45 samples from 19 counties (17 counties from Guilan province and 2 from the west of Mazandaran province) were collected and analysed (Figure 1). The collected samples were classified into four population groups: three population groups representing west, centre, and east of Guilan (WGP, CGP, EGP) and one population group representing the western part of Mazandaran province (WMP) (Figure 2). Sampling points were chosen according to the distribution of the rice cultivated areas in both provinces (Table 1).

DNA extraction. Genomic DNA was extracted from the larvae either using the DNA extraction Kit (DNA Extraction Kit DNP™; CinnaGen Inc., Tehran, Iran), or by employing the phenol-chloroform-isoamyl alcohol method (Sambrook *et al*. 1989). Extracted DNA was re-suspended in TE buffer (10mM Tris-HCl, pH 7.4, 1mM EDTA, pH 8.0) and stored at –20°C before amplification.

PCR amplification and agarose gel electrophoresis. After preliminary test with 15 randomly chosen primers (12 were synthesised in Faza Biotech Inc., Tehran, Iran and 3 – G13, 80.8, and 80.7 were synthesised in CinnaGen Inc., Tehran, Iran), 12 oligonucleotide primers were selected and the PCR amplification was performed. However three primers (OPB5, OPH3, and OPH9) were discarded later because OPB5 did not amplify any fragments and OPH3 and OPH9 produced only monomorphic bands. Each 25 µl PCR reaction mixture contained 50 ng of extracted DNA, 1 unit of Taq polymerase (CinnaGen Inc.), 0.22mM of dNTPs, 5 pmol of primer (Faza Biotech Inc., Teheran, Iran), 2.6mM of $MgCl_2$, and 1× reaction buffer (500mM KCl, 100mM TrisHCl, pH 9). Negative controls were employed in each PCR reaction. The PCR reaction performed on the DNA Thermal cycler (TGradient Module 48; Biometra GmbH, Göttingen, Germany) followed the following cycles: the initial denaturation step done at 94°C for 5 min was followed by 45 cycles consisting of a denaturation step at 94°C for 1 min by annealing at 36°C for 1 min, and by an extension step at 72°C for 2 minutes. After that, the last cycle lasting for 5 min at 72°C was used to allow complete build-up of any partially synthesised strand. The PCR products were size fractionated by electrophoresis on 1.5% agarose gel in 0.5× TBE buffer. The gels were stained with ethidium bromide (0.5 µg/ml) for about 20 min (Sambrook *et al*. 1989). A standard molecular weight marker (Mass ruler DNA ladder, Fermentas Life Sciences) was used in each electrophoretic run. The UV-transilluminated gels were photographed and analysed using Gel DOC 2000 Gel Documentation System and Quality One® software (Bio-Rad Laboratories, Hemel Hempstead, UK). Only unequivocally reproducible bands were scored, entered into a binary character matrix (1 for presence and 0 for absence of band) and used for data analysis.

Data analysis. In order to evaluate suitability of the used RAPD markers for exploration of genetic variation, polymorphic information content (PIC) was calculated for each primer according to the following formula: $PIC = 2\sum p_i q_i$ (where: p_i – frequency of 1 *i* band and q_i – frequency of 0 *i* band) and frequency of multi loci value (F_i) was calculated as $F_i = P_i/N$ (where P_i – number of individuals with *i* multi loci and *N* – total number of individuals). PIC and Fi values were measured for all primers separately in each population. Also the Nei's gene diversity (*h*) and Shannon index of diversity (Lewontin 1972) (I) were calculated by means of the POPGENE Version 1.32 software (Yeh *et al*. 1999). In order to describe the genetic variability among and within populations, the distribution of variance was analysed by performing analysis of molecular variance (AMOVA) (Excoffier *et al*. 1992) and the principal coordinate analysis (PCoA) (Orlóci 1978) by using GenAlEx Version 6.2 software (Peakall & Smouse 2006). For genetic distance analysis, POPGENE Version 1.32 and NTSYS-pc Version 2.02 software (Rohlf 2008) were used. The data were analysed by using SIMQUAL

Table 1. Population area and rice culture area extent in each county

Population	County	Rice culture area extent (ha)	No. of samples	Number of larvae	Sample	GPS coordinates for used samples	
Guilan West (WGP)	Astara	3 200	20	2	WGP1	38°25'38.74''N 48°51'38.20''E	–26 m
					WGP2	38°18'26.56''N 48°52'10.07''E	–19 m
	Talesh	4 800	20	2	WGP3	38°09'43.44''N 48°52'51.94''E	13 m
					WGP4	37°48'18.73''N 48°54'46.17''E	40 m
	Rezvan-shahr	9 300	20	2	WGP5	37°33'11.36''N 49°o7'41.88''E	17 m
					WGP6	37°35'44.08''N 49°04'15.12''E	23 m
	Masal	9 100	20	2	WGP7	37°21'41.47''N 49°07'23.78''E	66 m
					WGP8	37°24'40.56''N 49°07'01.18''E	62 m
Total extent (ha)	36 400	Total number of samples 80				Total number of larvae 8	
Guilan centre (CGP)	Anzali	4 200	20	2	CGP1	37°31'01.86''N 49°20'31.31''E	–27 m
					CGP2	37°27'05.29''N 49°29'02.82''E	62 m
	Somehsara	13 870	20	2	CGP3	37°21'03.39''N 49°12'52.31''E	5 m
					CGP4	37°17'55.84''N 49°18'08.69''E	6 m
	Fooman	27 150	20	2	CGP5	37°13'35.65''N 49°18'01.97''E	35 m
					CGP6	37°10'18.10''N 49°06'51.17''E	282 m
	Shaft	14 330	20	2	CGP7	37°09'43.75''N 49°24'19.03''E	47 m
					CGP8	37°08'47.89''N 49°20'54.75''E	74 m
	Rasht	62 000	50	7	CGP9	37°16'52.70''N 49°31'11.99''E	–2 m
					CGP10	37°18'04.96''N 49°33'21.59''E	–5 m
					CGP11	37°19'03.27''N 49°36'40.86''E	–8 m
					CGP12	37°17'33.49''N 49°37'53.03''E	–2 m
					CGP13	37°15'10.68''N 49°37'34.62''E	6 m
					CGP14	37°13'18.23''N 49°35'31.03''E	15 m
					CGP15	37°10'50.75''N 49°36'28.15''E	27 m
	Roudbar	3 500	20	3	CGP16	36°54'17.44''N 49°30'00.79''E	195 m
					CGP17	36°53'14.10''N 49°31'54.93''E	227m
					CGP18	36°39'35.15''N 49°27'02.72''E	750 m
	Siahkal	4 434	20	2	CGP19	37°07'57.01''N 49°52'18.94''E	82 m
					CGP20	36°53'09.50''N 49°54'23.39''E	1 545 m
Total extent (ha)	≈130 000	Total number of samples 170				Total number of larvae 20	
Guilan East (EGP)	Astaneh	20 100	20	2	EGP1	37°16'28.84''N 49°56'59.35''E	–11 m
	Lahijan	6 150	20	2	EGP2	37°14'54.64''N 49°56'33.93''E	–13 m
					EGP3	37°13'58.30''N 50°00'44.27''E	–14 m
	Langeroud	23 816	20	2	EGP4	37°10'46.35'N 49°58'55.31''E	13 m
					EGP5	37°12'28.20''N 50°09'47.06''E	–23 m
	Amlash	3 500	10	1	EGP6	37°10'2.62''N 50°09'45.00''E	–17 m
					EGP7	37°05'59.28''N 50°11'37.34''E	23 m
	Rodsar	107 00	20	2	EGP8	37°07'52.05''N 50°17'31.38''E	–19 m
					EGP9	37°03'0.24''N 50°19'18.91''E	32 m
Total extent (ha)	64 266	Total number of samples 90				Total number of larvae 9	
Mazandaran West (WMP)	Ramsar	–	40	4	WMP1	36°54'24.66''N 50°39'27.74''E	16 m
					WMP2	36°39'20.21''N 50°23'51.79''E	1 785 m
					WMP3	36°37'58.12''N 50°43'19.33''E	1 000 m
					WMP4	36°46'26.11''N 50°50'26.37''E	53 m
	Amol	38 450	40	4	WMP5	36°26'27.41''N 52°15'29.35''E	85 m
					WMP6	36°21'41.47“N 52°21'40.81''E	240 m
					WMP7	36°35'25.87''N 52°14'26.14''E	–17 m
					WMP8	36°29'41.41''N 52°24'57.29''E	49 m
Total extent (ha)	–	Total number of samples 80				Total number of larvae 8	
		Total number of samples 420				Total number of larvae 45	

Table 2. Measured PIC value for populations

Primer	Sequences	West (WGP)	Centre (CGP)	East (EGP)	West (WMP)
G13	CTC-TCC-GCC-A	0.18	0.25	0.21	0.16*
OPB14	TCC-GCT-CTG-G	0.24	0.24	0.31	0.29
OPF7	CCG-ATA-TCC-C	0.28	0.30	0.26	0.21
OPG11	TGC-CCG-TCG-T	0.32	0.43**	0.29**	0.43**
OPG16	AGC-GTC-CTC-C	0.25	0.27	0.37	0.22
H13	CTC-TCC-GCC-A	0.37**	0.36	0.32	0.16*
OPJ13	CCA-CAC-TAC-C	0.32	0.36	0.39	0.23
OPJ15	TGT-AGC-AGG-G	0.31	0.33	0.32	0.21
OPK3	CCA-GCT-TAG-C	0.37**	0.23*	0.32	0.28
OPK13	GGT-TGT-ACC-C	0.21	0.38	0.17*	0.22
80.7	GCA-CGC-CGG-A	0.36	0.27	0.19	0.25
80.8	CGC-CCT-CAG-C	0.10*	0.24	0.36	0.27
Min		0.28	0.31	0.29	0.24

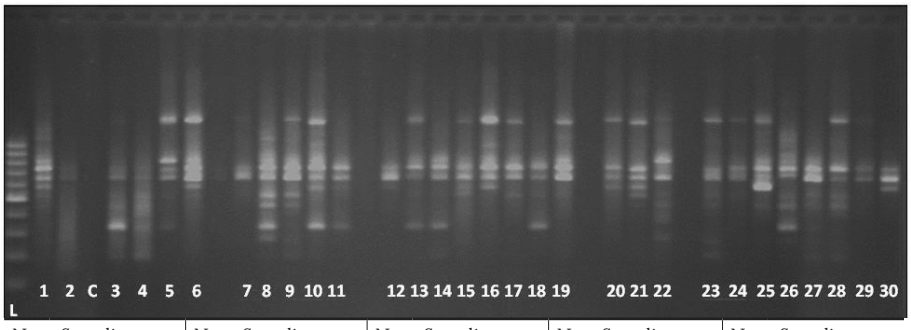
*lowest value; **highest value; PIC = $2\Sigma p_i q_i$; p_i = (1 – *i* band frequency); q_i = (0 – *i* band frequency) or (1 – p_i frequency)

(similarity for qualitative data) method to generate similarity/genetic distance among individuals using *Sm* coefficient (Sokal & Michener 1958). *Sm* coefficient was calculated from the following formula: $Sm = (N_a + N_d)/(N_a + N_b + N_c + N_d)$ by NT – sys Pc; where: $(N_a + N_d)$ – number of bands common to lanes a and d, N_a – total number of bands present in a; N_b – total number of bands in lane b; N_c – total number of bands present in c; N_d – total number of bands present in d (Nei & Li 1979)

The *Sm* similarity coefficients were used to construct a dendrogram using the unweighted pair group method with arithmetic averages (UPGMA) (Dunn & Everitt 1982) employing the SAHN (sequential, agglomerative, hierarchical, and nested clustering) module. To test the fitness of the UPGMA clustering, a co-phenetic (Ultrametric) value matrix was estimated from the Sm similarity matrix using COPH (Cophenetic values) (Rohlf & Sokal 1981). Statistical stability of the branches in the dendrogram was estimated by bootstrap analysis with 1000 replicates, using the WinBoot software program (Yap & Nelson 1996).

RESULTS

Pattern of variation in RAPD loci. During the DNA extraction protocol, special care was taken to minimise contamination with DNA from other organisms such as parasitoids. Nevertheless, despite the washing of samples by absolute ethanol, the presence of foreign DNA could not be absolutely ruled out due to a high prevalence of pathogens (e.g. fungi) in *Ch. suppressalis* populations, an aspect that has rarely been considered in other intra- and



No.	Sampling area	No.	Sampling area	No.	Sampling area	No.	Sampling area	No.	Sampling area
L	DNA ladder	6	CGP17	13	EGP9	20	EGP3	27	WMP4
C	Check	7	CGP18	14	CGP7	21	EGP4	28	WMP5
1	WGP5	8	WGP7	15	CGP8	22	EGP1	29	WMP6
2	EGP5	9	WGP8	16	WGP1	23	EGP2	30	WMP7
3	CGP3	10	WGP3	17	WGP2	24	WMP1		
4	CGP4	11	WGP4	18	CGP1	25	WMP2		
5	CGP16	12	EGP8	19	CGP2	26	WMP3		

Figure 3. Amplified pattern for some samples yielded by OPK3 primer

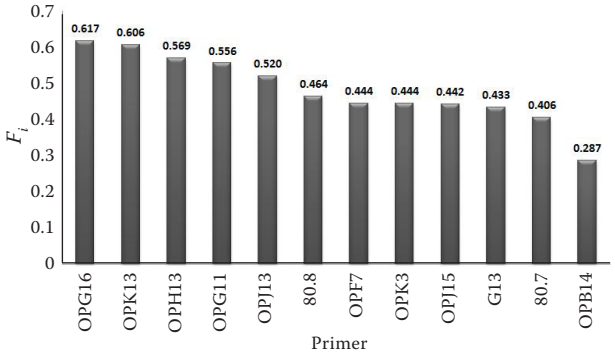


Figure 4. Multi loci values frequencies column chart

interspecific variability studies. All analysed primers yielded clear amplification patterns (Figure 3). A total of 114 fragments, sizing 164–1844 bp, were scored. Out of 114 loci, 112 fragments were polymorphic and 2 were monomorphic. The maximum and minimum PIC values (Table 2) for primers were 0.43 (representing primer OPG11) and 0.1 (representing primer 80.8). Frequency of multi loci value (F_i) (Figure 4) was determined among the tested primers, with OPG16 (0.617) and OPB14 (0.287) exhibiting maximum and minimum values, respectively. When genetic variation in Indian populations of *Scirpophaga incertulas* was studied by these primers (Kumar *et al*. 2001), Primer OPB14 followed by OPG16 produced the highest numbers of multi loci, which is comparable to our results.

RAPD banding patterns across populations (Table 3) showed the highest level of heterozygosity in CGP. There are two specific bands in CGP and one specific band in EGP and WMP that could distinguish these populations from each other. Also two locally common bands (frequency ≥ 5%) in WGP, seven in CGP, and five in EGP and four locally common bands in WMP were found in ≥ 50% of individuals in the populations (Table 3).

RAPD genetic diversity. Partitioning of molecular variance was calculated within and among populations. 59% of the total variability was attributable to differences among populations and 41% to differences within populations. The estimated numbers of observed alleles and effective alleles for all individuals were 1.98 and 1.51, respectively. Results indicated that CGP has the maximum number of observed and effective alleles. By calculating Nei's genetic variation and percentage of polymorphic loci for each population, CGP showed the highest level of genetic variation and the maximum percentage of polymorphic loci (Table 4). Maximum value of Shanon index determining diversity between population genotypes belonged to CGP as well.

Genetic distance and genetic similarity. Calculation of Nei's original measures of genetic identity and genetic distance (Table 5) showed that the WMP has maximum distance and minimum similarity with Guilan populations. However, results showed that the EGP has more similarity and shorter distance to WMP, and CGP is more similar to WGP. Results of clustering dendrogram (Figure 5) based on UPGMA method obtained by NTSYS-pc showed that there are differences among genetic structures of the studied populations. UPGMA cophenetic correlation value was 0.621. The studied samples could be divided into four groups representing the regions. 87% of the WMP samples were in group A and about 63% of the WGP samples were located in group B. These two regions were almost segregated from each other. 56% of the EGP samples were in group A and 44% in group D. CGP samples were distributed almost in all groups: 35% of them were in group A, 10% in B, 15% in C, and 40% in D group. Bootstrapping indicates that the robustness of the cluster was not very strong in some branches. Only a few branches with high (above 60%)

Table 3. Number of band patterns across populations

Populations	Guilan			Mazandaran West (WMP)
	West (WGP)	Centre (CGP)	East (EGP)	
No. bands	101	110	105	100
No. bands frequency ≥ 5%	101	110	105	100
No. private bands	0	2	1	1
No. L common bands (≤ 25%)	0	0	0	0
No. L common bands (≤ 50%)	2	7	5	4
Mean expected heterozygosity	0.2613 ± 0.02	0.2913 ± 0.02**	0.2787 ± 0.02	0.2292 ± 0.02*
Mean unbiased expected heterozygosity	0.2791 ± 0.02	0.2994 ± 0.02**	0.2957 ± 0.02	0.2449 ± 0.02*

*lowest value; **highest value; mean expected heterozygosity (He) = 2 × p × q; mean unbiased expected heterozygosity = (2N (2N – 1)) × He; for Diploid Binary data and assuming Hardy-Weinberg equilibrium, q = (1× band frequency)$^{0.5}$ and p = 1 – q

Table 4. Numbers of observed and effective alleles, estimated Nei's genetic variation, Shanon index, and percentage of polymorphic loci

Populations	Na	Ne	H	P (%)	I
WGP	1.71 ± 0.46	1.45 ± 0.38	0.26 ± 0.20	71.05	0.39 ± 0.28
CGP	1.90 ± 0.30	1.48 ± 0.33	0.29 ± 0.16	90.35	0.44 ± 0. 22
EGP	1.83 ± 0.37	1.46 ± 0.34	0.28 ± 0.17	83.33	0.42 ± 0.24
WMP	1.64 ± 0.48	1.39 ± 0.38	0.23 ± 0.20	64.91	0.34 ± 0.28
Total mean	1.98 ± 0.13	1.51 ± 0.31	0.31 ± 0.15	98.25	0.47 ± 0.18

Na – observed number of alleles; Ne – effective number of alleles; H – Nei's genetic diversity; P – percentage of polymorphic loci; I – Shannon Information index

Table 5. Nei's original measures of genetic identity and genetic distance

Genetic distance	Genetic similarity			
	WGP	CGP	EGP	WMP
WGP	****	0.9338	0.9291	0.9074
CGP	0.0684	****	0.9613	0.9101
EGP	0.0736	0.0395	****	0.9127
WMP	0.0972	0.0942	0.0914	****

*genetic similarities are shown above the diagonal and genetic distances are shown below the diagonal

bootstrap values were grouped. When the pattern of genetic variation and the impact of over-exploitation on the genetic structure of Turkish red pine (Kandemir *et al.* 2004; Lise *et al*. 2007) were studied with RAPD markers, the results were the same. Despite the low bootstrap values, a high genetic variation in red pine populations in the studied areas was found.

Principal coordinate analysis (PCoA). The results obtained using PCoA showed that the first four principal factors accounted for 14, 10, 10, and 8%, respectively, of the total variation (Figure 6). Despite these relatively low values, all populations are almost separated.

DISCUSSION

The primers used in this study varied widely in their ability to detect variation among populations. The OPF7 primer with 13 amplified fragments provided the maximum number of polymorphic bands and the largest polymorphic band was produced by this primer. Followed by primers OPF7, OPB14, and

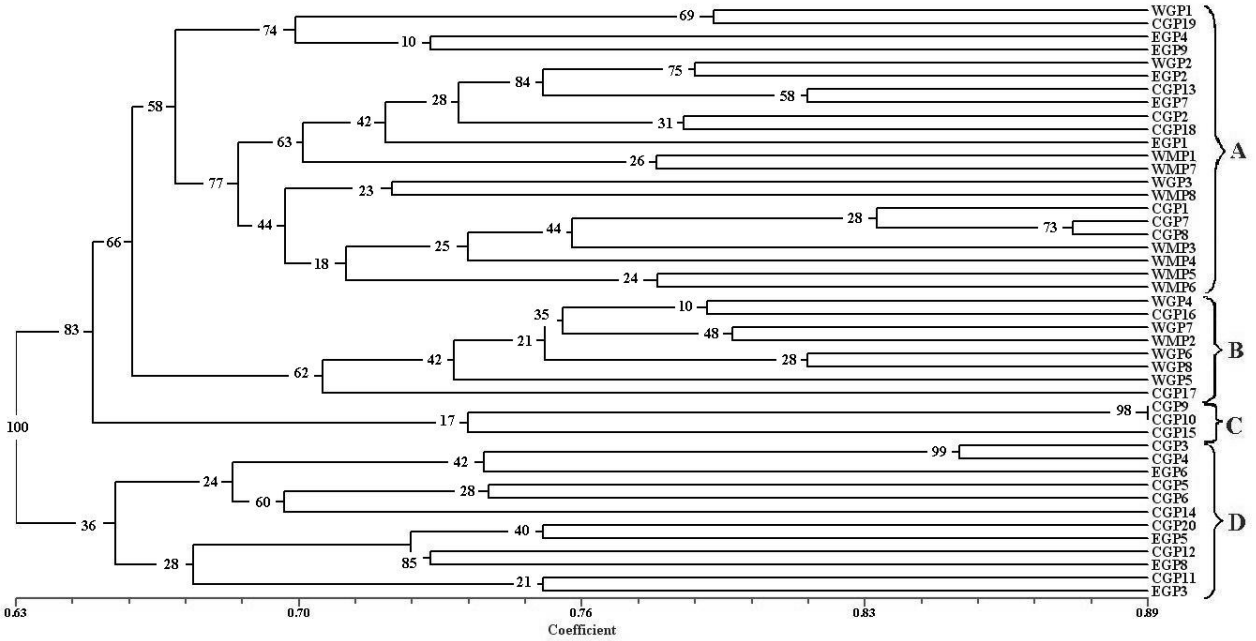


Figure 5. UPGMA dendrogram based on simple matching similarity coefficient among 45 *Chilo suppressalis* obtained from RAPD loci

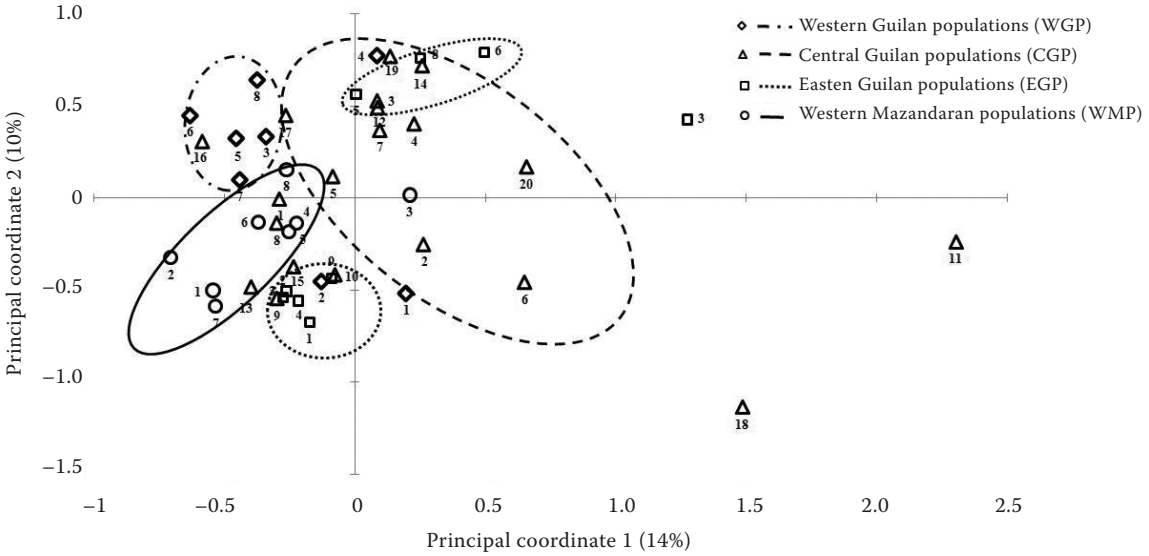


Figure 6. Principal coordination analysis based on the molecular banding patterns generated by RAPD analysis of 45 *Chilo suppressalis* individuals in Iran

OPK3 amplified the maximum number of polymorphic bands with 12 bands. OPK13 with 7 amplified bands produced the minimum polymorphic band. The estimated PIC value indicated that both H13 and OPK3 primers have the highest PIC value in the WGP. However, H13 in the WMP and OPK3 in CGP had the minimum values. The PIC was estimated for evaluation of suitability of the used RAPD markers and the results clearly showed occurrence of genetic differences among the investigated populations.

Genetic variation can be estimated as the number of heterozygous individuals in a population. For instance, Sosa-Gómez (2004) studied intra-specific variation and population structure of Velvetbean Caterpillar (*Anticarsia gemmatalis*) and found out that the population of the Velvetbean Caterpillar with the high level of heterozygosity exhibits the largest genetic variation. Studies on band patterns across populations (Table 3) showed the highest level of heterozygosity in CGP, as a result CGP can be considered as a population with the highest level of genetic variation. Similar results were obtained by estimates based on the number of observed alleles and effective alleles for all individuals, Nei's genetic variation, and Shanon index indicating the highest values for CGP. This result can be explained because the centre of Guilan province compared to other sampling regions comprises a wider rice cultivated area and the sample sizes collected from this region were larger in comparison to sample sizes from other regions. Loveless and Hamrick (1984) also proved that widespread species usually maintain a significantly high level of genetic diversity within populations and therefore extension of the area of activity increases the genetic variation level.

Results of AMOVA data analysis indicated a higher genetic variation among populations rather than within them. That is a contradictory result compared to the pattern frequently observed in both plants and animals (Moya *et al*. 2001). Low genetic variation within population could be due to the low distance between sampling areas and frequent gene flow between neighbouring populations.

Calculation of Nei's original measures of genetic identity and genetic distance (Table 5), clustering dendrogram (Figure 5) based on UPGMA method, and principal coordinate analysis (PCoA) (Figure 6) indicated that the studied regions could be divided into four groups but at this grouping populations were not completely separated from each other and estimated bootstrap values were low for some branches. Hampl *et al.* (2001) proved that the lower bootstrap values of the branches could be caused by the existence of a flow of genetic information among the strains of *Trichomonas vaginalis*. Some observations indicated that striped stem borer has the ability to fly over a distance of 5–10 miles and even farther if carried by winds (Pathak & Khan 1994). As a result, probability of gene flow can cause similarity and shorter genetic distance among these neighbourhood regions. So a possible reason for these results can be occurrence of gene flow among populations in each region. However, WMP has maximum distance and minimum similarity with Guilan populations

and EGP has more similarity and less distance to WMP. CGP was more similar to WGP. Calculated percentages of each population's samples appearance in each group of clustering dendrogram almost confirmed these results. Despite low values for the first four principal factors at PCoA, all populations are almost separated. Here, again WMP and WGP, with the longest geographical distance from each other, are classified into different groups

So all performed analyses almost separated *Ch. suppressalis* population into four distinct groups. However, as the cluster analysis showed, some individuals were not grouped with respect to their geographical origin due to a possible gene flow.

The phenotype and biochemical compounds of each individual could be the results of an interaction between its genotype and its environment (Armbruster *et al*. 2001). An understanding of evolutionary change occurring in agricultural systems could facilitate the formation and implementation of innovative strategies of pest management (Gould 1998). The results of this study provide evidence for genetic variability or evolution in general in response to ecological conditions of rice stem borer populations in the rice cultivated areas that could be useful in developing some strategies especially in the field of pesticide resistance management.

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