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Genetic diversity status of Pacific white shrimp (*Litopenaeus vannamei*) using SSR markers in Iran

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Abstract Genetic diversity is vital for the maintenance of genetic pool in cultured shrimps. In order to estimate the current status of genetic diversity in *Litopenaeus vannamei* shrimp in Iran, as an exotic species, a total of 45 individuals from Amiri and Gorgeaj farms in Jask port of Hormozgan province and one hatchery in Gomishan city of Golestan province, were detected using four microsatellite loci. The number of alleles per locus was 5–10, and the mean effective number of alleles (N_e) across populations and loci ranged from 4.834 to 5.148. The overall mean observed heterozygosity (H_o) ranged from 0.450 to 0.479, which was lower than the expected one (0.789–0.794). There was nothing remarkable about any of the allele frequencies across populations or loci. The mean inbreeding coefficient (F_{IS}) and pairwise genetic differentiation (F_{ST}) among populations were 41.6 % and 0.133, respectively. The three studied populations departed from Hardy–Weinberg equilibrium (HWE). Analysis of molecular variance revealed 14 % variability among and 86 % within populations. However, considering departing from HWE and the high F_{IS} and F, the moderate pairwise F_{ST} values, importance of introducing genetically diverse broodstock and monitoring to control inbreeding is discussed.

Keywords Genetic diversity · Microsatellite · *Litopenaeus vannamei*

Introduction

Genetic diversity is vital for the maintenance of genetic pool in cultured shrimps. But the production of postlarvae from crosses of breeders collected from an associated grow-out farm after mass selection, as a common practice in shrimp closed broodstock rearing systems,

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probably can lead to suffering from the effects of low genetic diversity caused by inadequate breeding strategies (Souza de Lima et al. 2008). In this process, the largest individuals are selected as prospective broodstock to produce the next generation. Indiscriminate selection of prospective broodstock can result in rapid loss of genetic diversity (Zhi-min et al. 2010). The loss of genetic diversity can compromise the effectiveness of selective breeding of *Litopenaeus vannamei* (De Donato et al. 2005; Zhi-min et al. 2010). Thus, it is important to monitor and control inbreeding (Norris et al. 2000) by increasing knowledge about parentage to ensure mating among unrelated individuals (Dong et al. 2006). Previous studies have demonstrated the effectiveness of using DNA microsatellite markers for managing genetic diversity and controlling inbreeding in farmed shrimp (Perez-Enriquez et al. 2009). Iran experienced heavy losses in shrimp production from 2001 to 2002 that have been attributed to white spot syndrome virus disease (WSSVD). In order to create diversity and introduce of more resistant species, a total of 80 pairs of *vannamei* imported from USA in 2003 (Afsharnasab et al. 2005). In the same year, the Iranian Fisheries Research Institute began doing research on *L. vannamei* shrimp and finally in 2004 achieved the techniques of breeding and culture of *L. vannamei* shrimp. Due to the lack of information about the stocks of *L. vannamei* that were imported into Iran, there is a need to assess the genetic diversity of these stocks.

The aim of this study was to use microsatellite markers to assess the genetic diversity of stocks of *L. vannamei* imported into Iran.

Materials and methods

Sample collection

A total of 45 individuals of *L. vannamei* shrimp in postlarval stage were sampled equally and randomly from two farms of Amiri and Gorgeaj in Jask port of Hormozgan province and one hatchery in the center of breeding and culture of *L. vannamei* in Gomishan city of Golestan province. Postlarvae were immediately fixed in 96° ethanol (Souza de Lima et al. 2008) and transferred to Laboratory of Biotechnology, Department of Fisheries, College of Agriculture and Natural Resources, Tehran University.

DNA extraction and microsatellite amplification

Genomic DNA was extracted from muscle and pleopods of sampled postlarvae using extraction kit of Bioneer Company (K-3032 AccuPrep® Genomic DNA Extraction Kit, Republic of Korea). Four previously described microsatellite markers TUDGLv5-7.33, TUDGLv7-9.17, TUDGLv1-3.224 (Garcia and Alcivar-Waren 2007), and $P_{van}1758$ (Cruz et al. 2004) have been chosen. Polymerase chain reactions were conducted in Gene Amp PCR system 9600 thermocycler under the following conditions: 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 44–60 °C for 1 min, and 72 °C for 1 min, followed by a final extension step of 72 °C for 10 min. Amplification volume was 25 µl with the following conditions: 50–150 ng of DNA, 1 µl (2.8 µM) of each primer, 12.5 µl of 2X Taq Master Mix of Vivantis Company, and 7.5 µl of distilled water.

Preparation of Metaphor agarose for banding resolution of PCR products

The PCR products for 4 amplifying loci were visualized on 3 % high-resolution Metaphor agarose gels (Cambrex Bio Science Rockland, Inc., USA) with ethidium (Grzybowski

2010) and analyzed (Image Analysis v1.0) to determine allele sizes and genotypes for each individual.

Data analysis

Polymorphic information content (PIC) has been analyzed using common GenAlEx 6.41 software (Peakall and Smouse 2006). Deviation from Hardy–Weinberg equilibrium (HWE) ($F_{IS} = 0$ or $F_{IS} \neq 0$), allele frequency, determination of homozygosity, observed heterozygosity (H_o), expected heterozygosity (H_e), and number of alleles (N_a) for each locus was calculated. Criteria such as F_{ST} and F_{IS} were used to estimate the value of gene flow (N_m) and inbreeding. Mean number of alleles (N_a), effective number of alleles (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e), Shannon index (sHua), polymorphic information content (PIC), and mean of Hardy–Weinberg equilibrium index (HWE) in four microsatellite loci in three populations of *L. vannamei* shrimp were analyzed by GenAlEx 6.41 population genetics software. Meanwhile, genetic distance (D) and genetic similarity coefficient (I) among populations were calculated by the Neis method (1972).

Results

Genetic diversity within and among populations

Genetic diversity of *L. vannamei* from three populations using four specific microsatellite loci of this species is shown in Table 1. Mean number of alleles (N_a) and mean effective number of alleles (N_e) in each population across the loci were 6.750–7.750 and 4.834–5.148, respectively. Numbers of alleles in each locus in TUDGLv5-7.33 locus with 8–10 alleles, followed by TUDGLv1-3.224 and TUDGLv7-9.17 loci with 5–10 and 6–8 alleles, and $P_{van}1758$ locus with 5–6 alleles were higher, respectively. Mean of observed heterozygosity (H_o) and expected heterozygosity (H_e) was 0.450–0.479 and 0.789–0.794, respectively. Among populations, N_a values ranged from 5 to 10, N_e values from 3.913 to 7.500, and H_o values from 0 to 0.800. In all cases, H_o values were lower than the values for H_e, except for TUDGLv1-3.224 locus in Gorgeaj population, indicating a general deficit of heterozygous types for the under-studied loci (Table 1).

Also, fixation index in these three populations was 0.413, 0.418, and 0.438, in Amiri, Gorgeaj, and Gomishan populations, respectively. Mean PIC across three populations in each locus ranged from 0.88 to 0.92. This showed that four loci had high information content in *L. vannamei* samples. In particular, TUDGLv5-7.33 had highest, while TUDGLv1-3.224 had lowest polymorphism. Results of pairwise population analysis for Shannon index (sHua) and gene flow (N_m) that are indices of genetic diversity within and among populations, across the three populations and loci, were significant ($P < 0.001$), indicating the existence of genetic variation within populations and insufficient migration among populations to oppose the drift effect (Table 2).

Allele size range (bp) in Amiri, Gorgeaj, and Gomishan populations across the four surveyed loci ranged 120–230, 120–225 and 105–220, respectively. With considering the number of alleles observed across the loci and populations, a total of 70 genotypes were found in the study of these three populations. TUDGLv7-9.17 locus in Amiri population with 13 types of genotypes and TUDGLv5-7.33 and TUDGLv1-3.224 loci with 12 types

Table 1 Genetic diversity found in three populations of the *L. vannamei* shrimp in four loci

Population		Locus				Mean
		TUDGLv5-7.33	TUDGLv7-9.17	TUDGLv1-3.224	$P_{van}1758$	
Amiri	N	13	15	15	14	14.250
	N_a	8	10	8	5	7.750
	N_e	4.333	7.500	4.839	3.920	5.148
	I	1.777	2.153	1.775	1.470	1.794
	H_o	0.385***	0.800	0.733	0.000***	0.479
	H_e	0.769	0.867	0.793	0.745	0.794
Gorgeaj	N	14	15	15	15	14.750
	N_a	8	8	8	6	7.500
	N_e	4.170	5.488	4.787	4.891	4.834
	I	1.735	1.858	1.805	1.662	1.765
	H_o	0.500**	0.333***	0.800	0.200***	0.458
	H_e	0.760	0.818	0.791	0.796	0.791
Gomishan	N	15	15	15	15	15
	N_a	10	5	6	6	6.750
	N_e	7.500	3.913	4.455	4.369	5.059
	I	2.137	1.468	1.619	1.609	1.708
	H_o	0.600	0.133***	0.733	0.333***	0.450
	H_e	0.867	0.744	0.776	0.771	0.789

N number of individuals, N_a number of alleles, N_e number of effective alleles, I Shannon's information index, H_o observed heterozygosity, H_e expected heterozygosity

Significance of Hardy–Weinberg departure: ** $P < 0.01$; *** $P < 0.001$

Table 2 Results of pairwise population analysis for Shannon index (sHua) and gene flow (N_m), for codominant data in each locus

Population	Locus	sHua	N_m	P	Sig
Amiri–Gorgeaj	TUDGLv5-7.33	0.858	0.033	0.000	***
Amiri–Gomishan		0.860	0.033	0.000	***
Gorgeaj–Gomishan		0.785	0.039	0.000	***
Amiri–Gorgeaj	TUDGLv7-9.17	0.600	0.068	0.000	***
Amiri–Gomishan		0.765	0.042	0.000	***
Gorgeaj–Gomishan		0.845	0.034	0.000	***
Amiri–Gorgeaj	TUDGLv1-3.224	0.560	0.078	0.000	***
Amiri–Gomishan		0.470	0.110	0.000	***
Gorgeaj–Gomishan		0.554	0.079	0.000	***
Amiri–Gorgeaj	$P_{van}1758$	0.895	0.030	0.000	***
Amiri–Gomishan		0.705	0.049	0.000	***
Gorgeaj–Gomishan		0.624	0.062	0.000	***

sHua Shannon's mutual information index, N_m number of migrants per generation, *Sig* significance of allele frequency differences: *** $P < 0.001$

of genotypes in Gomishan and Gorgeaj populations, respectively, had highest, and $P_{van}1758$ locus in Amiri population with five types of genotypes had lowest genotype diversity. The genotypes of FF in $P_{van}1758$ and TUDGLv7-9.17 loci of Amiri and Gomishan populations, respectively, and II in TUDGLv5-7.33 and KK in $P_{van}1758$ loci of Amiri and Gomishan populations, respectively, with five repeats, had the highest frequency

(Table 3). Amiri population in TUDGLv5-7.33 and $P_{van}1758$ ($P < 0.001$) loci; Gorgeaj population in TUDGLv5-7.33 ($P < 0.01$), TUDGLv7-9.17, and $P_{van}1758$ ($P < 0.001$) loci; and Gomishan population in TUDGLv7-9.17 and $P_{van}1758$ ($P < 0.001$) loci had deviation from Hardy–Weinberg equilibrium (HWE). In other loci, these three populations were in Hardy–Weinberg equilibrium ($P > 0.05$) (Table 4). In total, 43 types of alleles were found in these populations and loci surveyed. The analysis of allele frequency showed that there was no common allele with remarkable frequency. Also, the alleles of 150, 134, and 132 bp in TUDGLv5-7.33 locus of Amiri, Gorgeaj, and Gomishan populations with the frequency of 0.423, 0.429, and 0.200 were exclusive and had a remarkable frequency, respectively. In this locus, five exclusive alleles in Amiri–Gorgeaj and seven exclusive alleles in Gomishan populations were found, respectively. The alleles of 184 bp in TUDGLv7-9.17 locus of Amiri population, 122, 152 and 155 bp, 162 bp in Gorgeaj and Gomishan populations of this locus with the frequency of 0.233, 0.200, 0.300, and 0.267, 0.367 had a remarkable frequency, respectively. In this locus, three exclusive alleles in Amiri and two exclusive alleles in Gorgeaj–Gomishan populations were found, respectively. In TUDGLv1-3.224 locus, the alleles of 210, 225 bp in Amiri, 192, 217 bp in Gorgeaj, and 175, 210, 220 bp in Gomishan populations, with the frequency of 0.233, 0.333, 0.200, 0.367 and 0.200, 0.333, 0.233, had a remarkable frequency, respectively. In this locus, four exclusive alleles in Amiri population, three exclusive alleles in Gorgeaj, and two exclusive alleles in Gomishan populations were found. The alleles of 184 and 188 bp in $P_{van}1758$ locus of Amiri population with the frequency of 0.357, 0.286 and also the alleles of 155, 170 and 165, 172 bp in Gorgeaj and Gomishan populations with the frequency of 0.200, 0.300 and 0.333, 0.267 had a remarkable frequency, respectively. In this locus, three exclusive alleles in Amiri–Gorgeaj populations and two exclusive alleles in Gomishan population were found (Fig. 1). All analyzed populations showed a total of positive values during the estimation of F_{IS} test, with mean of 0.431. It means that there were complete reductions of heterozygous in the three under-studied populations. Likewise, this issue was observed in the three under-studied loci, except for TUDGLv1-3.224 (Table 5). In locus of TUDGLv1-3.224, due to the less difference between H_o and H_e values in general and especially the higher H_o than H_e value in TUDGLv1-3.224 locus of Gorgeaj population, the estimated F_{IS} value for this locus was lower than the other three studied loci. Estimating of pairwise F_{ST} values, among the studied populations, indicated moderate genetic differentiation. On the other hand, pairwise N_m values obtained, indicated adequate gene flow among three populations. The F_{ST} values were significantly different ($P < 0.01$) (Table 6). Coefficient of genetic identity among Amiri–Gorgeaj, Amiri–Gomishan, and Gorgeaj–Gomishan was 0.149, 0.248, and 0.193, respectively. In particular, coefficient of pairwise genetic identity of Amiri–Gomishan and Amiri–Gorgeaj was highest and lowest, respectively. Pairwise genetic distance among Amiri–Gorgeaj, Amiri–Gomishan, and Gorgeaj–Gomishan was 1.902, 1.394, and 1.647, respectively. In particular, pairwise genetic distance of Amiri–Gorgeaj and Amiri–Gomishan was highest and lowest, respectively.

Analysis of molecular variance

Three studied populations had high and low genetic diversity within and among populations, respectively (Fig. 2). Statistics of PhiPT and coefficient of gene flow (N_m) calculated by the estimation of molecular variance based on genotypic distance indicated that moderate genetic differentiation and adequate gene flow are existed among surveyed populations (Table 7).

Table 3 Number of alleles (N_a) and allele size range (bp) in each locus of population

Population	Locus	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
Amiri	TUDGLv5-7.33	194	–	184	–	–	–	–	152	150	147	–	–	138	–	–	128	–	120	–	–	–
	TUDGLv7-9.17	184	180	176	170	165	162	–	–	152	–	145	139	–	–	105	–	–	–	–	–	–
	TUDGLv1-3.224	230	225	–	–	210	200	194	–	–	180	175	–	–	160	–	–	–	–	–	–	–
	P_{van}1758	–	–	–	188	–	184	176	172	–	167	–	–	–	–	–	–	–	–	–	–	–
Gorgeaj	TUDGLv5-7.33	–	189	–	178	172	–	–	–	–	–	144	–	–	134	–	128	125	120	–	–	–
	TUDGLv7-9.17	–	–	–	–	165	–	160	155	152	–	145	139	122	–	105	–	–	–	–	–	–
	TUDGLv1-3.224	–	225	–	217	210	200	–	192	182	–	175	167	–	–	–	–	–	–	–	–	–
	P_{van}1758	199	196	–	–	186	–	176	–	170	–	–	155	–	–	–	–	–	–	–	–	–
Gomishan	TUDGLv5-7.33	–	–	–	–	–	162	155	–	–	–	–	142	138	–	132	–	125	120	115	110	105
	TUDGLv7-9.17	–	–	–	170	–	162	–	155	–	150	–	–	–	120	–	–	–	–	–	–	–
	TUDGLv1-3.224	–	–	220	–	210	200	–	192	–	–	175	–	165	–	–	–	–	–	–	–	–
	P_{van}1758	199	–	192	–	–	–	176	172	–	–	165	155	–	–	–	–	–	–	–	–	–

Table 4 Summary of Chi-square tests for Hardy–Weinberg equilibrium (HWE)

Population	Locus	*df*	Chi-square	*P*	Sig
Amiri	TUDGLv5-7.33	28	57.885	0.001	***
	TUDGLv7-9.17	45	57.463	0.101	ns
	TUDGLv1-3.224	28	28.441	0.441	ns
	$P_{van}1758$	10	56.000	0.000	***
Gorgeaj	TUDGLv5-7.33	28	50.944	0.005	**
	TUDGLv7-9.17	28	76.157	0.000	***
	TUDGLv1-3.224	28	22.920	0.737	ns
	$P_{van}1758$	15	46.719	0.000	***
Gomishan	TUDGLv5-7.33	45	51.417	0.237	ns
	TUDGLv7-9.17	10	46.525	0.000	***
	TUDGLv1-3.224	15	17.934	0.266	ns
	$P_{van}1758$	15	44.838	0.000	***

df degree of freedom, *P* probability, *Sig* significance of Hardy–Weinberg departure, *ns* nonsignificant

** $P < 0.01$; *** $P < 0.001$

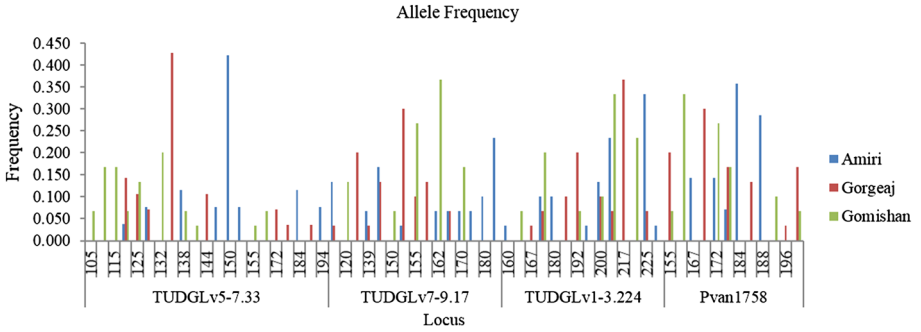

Fig. 1 Allele frequencies across populations and loci. Forty-three types of alleles were found across populations and loci

Table 5 *F* statistics values across populations for each locus

Stat	TUDGLv5-7.33	TUDGLv7-9.17	TUDGLv1-3.224	$P_{van}1758$	Mean	*P*
F_{ST}	0.148	0.117	0.117	0.148	0.133	0.01**
F_{IS}	0.381	0.505	0.074^{ns}	0.762	0.431	0.01**

F_{ST}, $(H_t - \text{mean } H_e)/H_t$; H_t, total expected heterozygosity; mean H_e, average H_e across the populations; F_{IS}, (mean H_e − mean H_o)/mean H_e; mean H_e, average H_e across the populations; mean H_o, average H_o across the populations; *P,* probability, *ns*, nonsignificant

** $P < 0.01$

Discussion

The specific loci were selected from the literature as the most highly diverse for this species (Cruz et al. 2002, 2004; Valles-Jiménez et al. 2005; Machado-Tamayo 2006; Garcia and Alcivar-Waren 2007; Luvesuto et al. 2007). We had 6 and 4 % reduction of H_o in Amiri–Gomishan (0.479–0.450) and Amiri–Gorgeaj (0.479–0.458) populations that represent a 5.4 and 3.6 % increase in inbreeding, respectively. Also, based on loci, we had

Table 6 Pairwise F_{ST} and N_m estimates among three populations of *L. vannamei*

Population 1	Population 2	F_{ST}	N_m	*P*
Amiri	Gorgeaj	0.141	1.527	0.01**
Amiri	Gomishan	0.123	1.784	0.01**
Gorgeaj	Gomishan	0.134	1.611	0.01**

F_{ST}, $(H_t - \text{mean } H_e)/H_t$; H_t, total expected heterozygosity; mean H_e, average H_e across the populations; N_m, number of migrants per generation; *P*, probability

** $P < 0.01$

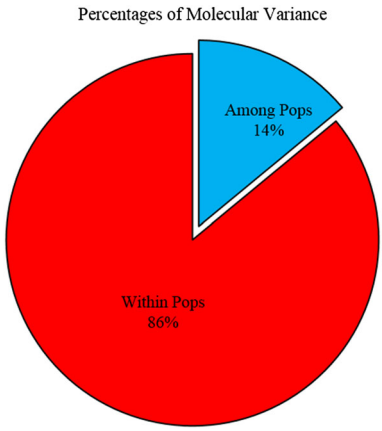

Fig. 2 Molecular variance within and among populations %

Table 7 PhiPT statistics indicating genetic differentiation

Stat	Value	*P*
PhiPT	0.140**	0.01
Nm	1.535	

AP estimation of variance among populations, *WP* estimation of variance within populations, N_m number of migrants per generation, *P* probability

** $P < 0.01$; PhiPT = AP/(WP + AP)

a remarkable reduction (76.45 %) of H_o in TUDGLv1-3.224–P_{van}1758 loci that represent a 68.8 % increase in inbreeding (Perez-Enriquez et al. 2009). In this study, mean polymorphic information content (PIC) 0.90, indicated the highly polymorphic nature of the under-studied loci, that can express the number of parents involved in the formation of the next generation is adequate. This means that the selection genetic diversity in terms of allelic diversity will maintain at original varieties level (Zhi-min et al. 2010). In P_{van} 1758 locus, N_a was lower and N_e, H_o, and H_e were in the range of Perez-Enriquez et al. (2009). In this study, mean number of alleles by loci 5.667–8.667 was similar to Cruz et al. (2004). Based on the number of alleles as a main index of genetic diversity, we had reduction of 12.9 % of N_a in Amiri population (7.75) to Gomishan population (6.75) (Perez-Enriquez et al. 2009). In *L. stylirostris,* a consistent and progressive reduction in heterozygosity levels was attributed to a severe bottleneck effect in the founder population (Machado-

Tamayo 2006). In tropical species such as *L. vannamei,* N_m values higher than 1, as showed in the results based on genetic differentiation that it is dependent on heterozygosity and allele frequency and is one of the indices of genetic diversity among populations, are necessary for the maintenance of genetic diversity and heterozygosity and against random genetic drift which tends to make populations genetically more heterogeneous (Oliveira et al. 2006). However, for all of the pairwise populations per each locus, because of the low gene flow, there were high significant differences in Shannon's index, as one of the indices of genetic diversity within population is based on mean of allele frequency and number of alleles. This issue can be achieved from difference in allele frequency that was significant in mentioned loci and populations (Oliveira et al. 2006). Therefore, as mentioned above, existence of adequate *N*m, especially in closed rearing populations, with the assumption of high genetic diversity of main founder population, is necessary for the maintenance of genetic diversity. The allele size range in three loci except for $P_{van}1758$ indicated the high level of genetic diversity despite breeding in capture conditions (Garcia and Alcivar-Waren 2007). In these three loci, the allele size range differences based on loci were 50–79, that is similar to values obtained by Garcia and Alcivar-Waren (2007) in cultured *L. vannamei* shrimps. Deficits of heterozygotes cause deviations from Hardy–Weinberg equilibrium (Machado-Tamayo 2006). In this study, three of four loci: Amiri population in TUDGLv5-7.33 and $P_{van}1758$ ($P < 0.001$) loci; Gorgeaj population in TUDGLv5-7.33 ($P < 0.01$), TUDGLv7-9.17, and $P_{van}1758$ ($P < 0.001$) loci; and Gomishan population in TUDGLv7-9.17 and $P_{van}1758$ ($P < 0.001$) loci had deviations from Hardy–Weinberg equilibrium. In loci that deviation from Hardy–Weinberg equilibrium was observed, we had deficits of H_o relative to H_e. According to the Zhi-min et al. (2010), heterozygosity (*H*), also known as gene diversity, is the best parameter for the measurement of population genetic variation. In this study, mean heterozygosity of *L. vannamei* populations was 0.7–0.9, which indicates that the three studied populations have rich genetic diversity based on allelic variation. In the loci of TUDGLv5-7.33, TUDGLv1-3.224, TUDGLv7-9.17, and $P_{van}1758$, five, four, three, and one alleles with low frequency (0.01–0.04) were observed, respectively. The frequency of some alleles was zero (0.000); thus, the high risks of homozygosity are probable (Garcia and Alcivar-Waren 2007). With considering the existence of common alleles among these populations, we can nearly express the common origin of surveyed samples (Perez-Enriquez et al. 2009). Also, the existence of exclusive alleles can be the index of individual diversity (Machado-Tamayo 2006). In this study, mean of inbreeding coefficient (F_{IS}), based on loci and populations, was 43.1 and 41.6 %, respectively. The value of fixation index (*F*) in the locus of TUDGLv1-3.224 of Gorgeaj population was −0.011, indicating the excess of heterozygosity ($H_o > H_e$) (Souza de Lima et al. 2008). Deviation from Hardy–Weinberg equilibrium as observed by inbreeding coefficient is due to the deficit of heterozygous. Heterozygosity deficiency can be the result of the failure to amplify one of the alleles (Machado-Tamayo 2006). Also, Wahlund effect, i.e., subdivision of the local population into isolated and differentiated reproductive units, causes the shortage of heterozygous (Machado-Tamayo 2006). It has been demonstrated that penaeids can tolerate an inbreeding of 28 and 32–80 %. Nevertheless, Moss et al. (2007) recommended the inbreeding did not go over 10 % (Perez-Enriquez et al. 2009). The smaller the population genetic distance is, the shorter the differential time is, the closer the genetic relationship is, the lower the genetic variation is, and the larger the similarity coefficient is (Zhi-min et al. 2010). In our study, pairwise F_{ST} values among populations were 0.123–0.141, with the mean of 0.133, indicating that in spite of adequate pairwise gene flow (N_m) (Oliveira et al. 2006), which leads to the homogenization of allelic frequencies, moderate genetic differentiation exists among studied populations (Zhi-min et al.

2010). On the other hand, with considering the values obtained by analysis of molecular variance (AMOVA), based on genotypic (PhiPT) and allelic (F statistics) distances, 0.140 and 0.133, respectively, that indicating moderate genetic differentiation among the three studied populations (Zhi-min et al. 2010), we can express that in spite of the high values for F_{IS}, we have a remarkable genetic diversity among the studied populations.

Conclusions

In the final summation, PIC and Shannon indices represent the well-allelic status. High F_{IS} and moderate F_{ST} highlight the importance of constant evaluation of genetic diversity in cultured populations of *L. vannamei* in Iran. The high heterozygosity >0.5 means that these studied populations have a rich genetic diversity. However, in limited number of loci, zero H_o, and thus maximize F (1), also, very low frequency of some alleles (0.000), indicating the high risks of homozygous, and the need to manage broodstocks by aspect of maintaining genetic diversity indices and avoiding of genetic erosion are of great importance. Based on above-mentioned indices within and among populations, N_a, PIC, N_e, I, sHua, H_o, H_e, and AMOVA, F statistics, respectively, and with considering departing from HWE and the high F_{IS} and F, necessity for the existence of adequate gene flow within and among current populations and introducing broodstocks with rich genetic pool, with regard to hygienic conditions from the perspective of preventing the entry of pathogens, and cross-breeding among populations, in order to maintenance genetic diversity and reduce the risk of inbreeding depression, further will be revealed.

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