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Detection of Genetic Variation and Genetic Diversity of Common Carp in Sulaimani Governorate Using RAPD- PCR Technique

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Abstract: RAPD-DNA markers were used to study genetic diversity between seven samples of common carps from two lakes (Dukan and Darbandikhan) in Sulaimani governorate. A total of 21 samples were typed using twenty RAPD primers. Sixteen primers had clear bands, which used to investigate the genetic diversity and variation among seven samples of common carps. A total of 221 bands were scored, of which 116 bands (52.48%) were polymorphic and 61 of polymorphic band were unique bands. For all samples, Nei's gene diversity averaged 0.3214. Using weighted pair-group method with arithmetic average (UPGMA) dendrograms, the six clusters, the 1st cluster branch consisted of the type 3 and 4, the 2nd cluster was including type 6, the 3rd cluster including only type 2, the 4th cluster including type 1, the 5th cluster including type 7 and the 6th cluster including type 5. These results indicated that the type 1 is most genetically distant from the type 5 (0.9808). The types 3 and 4 in the 1st cluster indicating a close relationship between them and the results indicated that the fishes in type 3 and 4 were closer to type 6. The dendrograms showed that there is genetic diversity among seven fish samples, which ranged from 0.1335 to 0.9808. Based on the high degree of genetic distance between the seven fishes for the two lakes (Dukan and Darbandikhan), it is concluded, that the seven fishes are different from each other and this result showed the effect of lakes on genetic diversity in these common carps.

Key words: Common carp fish, genetic diversity and variation, RAPD PCR.

INTRODUCTION

Cyprinus carpio (family Cyprinidae) contributes over 20 million metric tons to fish production worldwide and accounts for approximately 40% of the total global aquaculture production and 70% of the total freshwater aquaculture production. They have emerged as the most economically important teleost family. In comparison to other major aquaculture species, such as salmon and shrimp, carps are recognized as eco-friendly fishes because most of them are omnivorous filter-feeders and thus consume much less fish meal and fish oil. As one of the dominant cyprinid species, *C. carpio* is cultured in over 100 countries worldwide and accounts for up to 10% (over 3 million metric tons) of global annual freshwater aquaculture production (FAO, 2007) and one of the most important fish species in aquaculture (Yousefian & Laloei, 2011) has been a popular aquaculture fish for more than 2,000 years.

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C. carpio is one of the most commercially important and widely cultivated freshwater fish in the world and contributes to 11% of the total world freshwater aquaculture production (Krouma, 2011). More than 90% of this fish production comes from Asia where this fish is cultured in various pond aquaculture systems. Similarly, common carp might alter its food preference and behavior in response to changing food resources (Adamek et al., 2003; Rahman et al., 2006; 2008).

Common carp varieties (eg. races, landraces, strains, breeds and stocks) have been developed through a combination of conditions including geographical isolation, adaptation, accumulation of mutations and naturals as well as human selection pressure (Jewel et al., 2006).

In the 1950s, carp species were introduced for the first time in Iraq, but only for scientific research purposes. The main aim was to acclimatize these species in the Iraqi inland waters and to establish whether they would be suitable for rearing in the Iraqi environment without interference and without a negative impact on endemic species. However, at that time, this experience was not channeled into commercial activities. Later on, a significant attention has been given to the aquaculture sector, initially with the establishment of hatcheries and the construction of fish farms (FAO, 2016).

Genetic variations of common carp population or strains have been investigated by scientists using different molecular markers. Recently, allozyme and microsatellite were used for Dutch carp (Tanck et al., 2000), French and Czech carps (Desvignes et al., 2001), transferrin marker, RAPD and microsatellites for Hungarian carp (Bártfai et al*.,* 2003) and Chinese carp (Liao et al., 2006; Yousefian & Laloei, 2011).

Random amplified polymorphic DNA (RAPD) analysis is a technique based on the polymerase chain reaction (PCR) amplification of discrete regions of genome with short oligonucleotide primers of arbitrary sequence (Welsh & McClelland, 1990; Williams et al., 1990). The method is simple and rapid technique for determining genetic diversity, variation and no prior knowledge of the genome under study is required (Hadrys et al., 1992). RAPD analysis also has been used to evaluate genetic diversity for species and subspecies identification in guppy (Dinesh et al., 1993), Tilapia (Bardakci & Skibinski, 1994; Dinesh et al., 1996), brown trout and Atlantic salmon (Elo et al., 1997), largemouth bass (Williams et al., 1998), Indian major carps (Barman et al., 2003) and damsel fishes (Parveen et al., 2011). In this study, the genetic variation and genetic diversity will be analyzed in common carp using RAPD marker.

MATERIALS AND METHOD

Sampling and DNA extraction

Blood samples of 21 wild common carps were collected in 10 mL vacationer tubes containing the anticoagulant, Ethylenediaminetetraacetic Acid (EDTA) from two lakes (1-4 from Dukan and 5-7 from Darbandikhan) in Sulaimani governorate. The three samples for each type were mixed together to make one sample (pooled sample). Genomic DNA was extracted from each of the blood sample using AccuPrep® Genomic DNA extraction Kit (Bioneer Corporation Cat. No.: K-3032 Korea). Quality of DNA was determined using 1% agarose gel electrophoresis.

RAPD primer

In the present study, a total of 20 RAPD primers were obtained from CinnaGen Inc. The description of primers regarding their names, primer sequences and GC percentages was given in Table (1).

PCR amplification

Amplifications of DNA were performed using a thermal cycler with the final reaction volume of 25 µL. Each reaction volume contained 12.5 µL of one PCR master mix, 2µL of RAPD primer 3 µL of DNA template and 7.5 µL of water free DNase. Three different protocols were used; the 1st Primers S-71, S-111, S-131, S-145, S-159, S-161, S-177, S-187, C04, C06, C07, C08, C09 and C011 was programmed for 45 cycles, initial denaturation of 1 min at 94 °C, denaturation at 94 °C for 1 min, annealing at 36 °C for 1 min, extension at 72 °C for 1 min and final extension of 5 min at 72 °C. The 2nd protocol for Primers P10, P8 and P5 was programmed for 45 cycles, initial denaturation of 2 min at 94 °C, denaturation at 94 °C for 30 sec, annealing at 40 °C for 30 sec, extension at 72 °C for 30 sec and final extension of 10 min at 72 °C. The 3rd protocol for Primers P7, P29 and R45 was programmed for 45 cycles, initial denaturation of 5 min at 94 °C, denaturation at 94 °C for 2 min, annealing at 45 °C for 1 min, extension at 72 °C for 2 min and final extension of 10 min at 72 °C. The amplification products were size-fractionated in a 1.5% agarose gel containing ethidium bromide in Tris-borate EDTA buffer and visualized under UV transillumination.

Genotypic analysis

The RAPD bands were scored as present (1) or absent (0) in each pattern. All genetic parameters in the present study were calculated by using POPGENE software version 1.32 (Yeh & Boyle, 1997).

Table 1. Primers, sequences and GC percentages.

	Primer name	Sequence 5⟶3	GC%
1	S-71	AAA GCT GCG G	60
2	S-111	AAT CGG GCT G	60
3	S-131	GGT CCC TGA C	70
4	S-145	GAC GGA TCA G	60
5	S-159	ACG GCG TAT G	60
6	S-161	TGC CGA GCT G	70
7	S-177	CAG GCC CTT C	70
8	S-187	AGT CAG CCA C	70
9	C04	CCG CAT CTA C	60
10	C06	GAA CGG ACT C	60
11	C07	GTC CCG ACG A	70
12	C08	TGG ACC GGT G	70
13	C09	CTC ACC GTC C	70
14	C011	AAA GCT GCG G	60
15	P10	AGC AGG TGG A	70
16	p7	CTG AGG AGT G	60
17	P8	GGG CTA GGG T	70
18	P5	GAA TGC GAC G	60
19	P29	CCG GCC TTA C	70
20	R45	GCC GTC CGA G	80

RESULTS

Total fragment numbers (TFN)

Out of the twenty primers which were used in this study, sixteen primers showed clear bands and applied to investigate the genetic variations among the common carps (Figs. 1- 4). The TFN for the 16 primes over all the fish samples was 221 fragments, ranged from 2 fragments in C04 to 36 fragments in P10 (Table 2). The size range of fragments for 16 primers over all the fish samples, ranged from 120 to 3000 bp, while the size range of fragments ranged from 200 to 3000, 150 to 2000, 220 to 1250, 200 to 1500 bp, 120 to 1000, 310 to 350 and 250 to 1500 in 1, 2, 3, 4, 5, 6 and 7 fish samples, respectively (Table 2). This result demonstrated a high differences between fish samples, especially between sample 1 and sample 5.

Polymorphic fragment numbers (PFN)

A total of 116 polymorphic fragments were obtained out of 221 TFN from 16 primers (Table 3). The highest PFN was found at locus S-161 (14 bands), whereas the lowest PFN was found at locus C04 (2 bands).

Unique of band

Table (4) showed that out of 221 bands overall fish samples, 61 were unique bands. The highest unique bands were obtained from C09 and S-159 locus which have 8 unique bands. Therefore, S-71 and S-177 showed one unique band. These results indicated that these loci can be used to analyze the genetic variation between fish samples.

Nei's gene diversity

The Nei's gene diversity (gene diversity/ heterozygosity) overall fish samples averaged 0.3214 (Table 5).

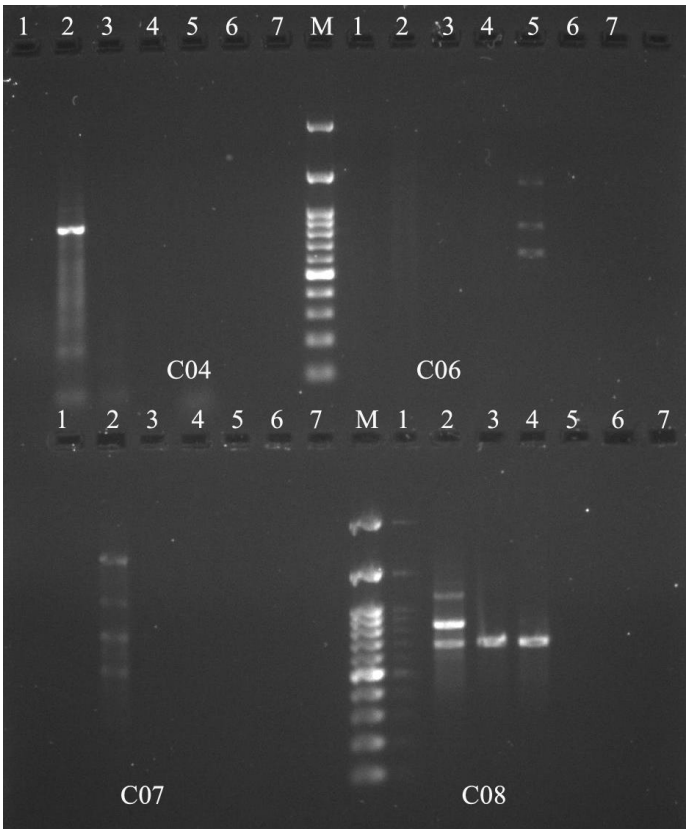

Figure 1. Gel electrophoresis for four RAPD primers of seven common carps.

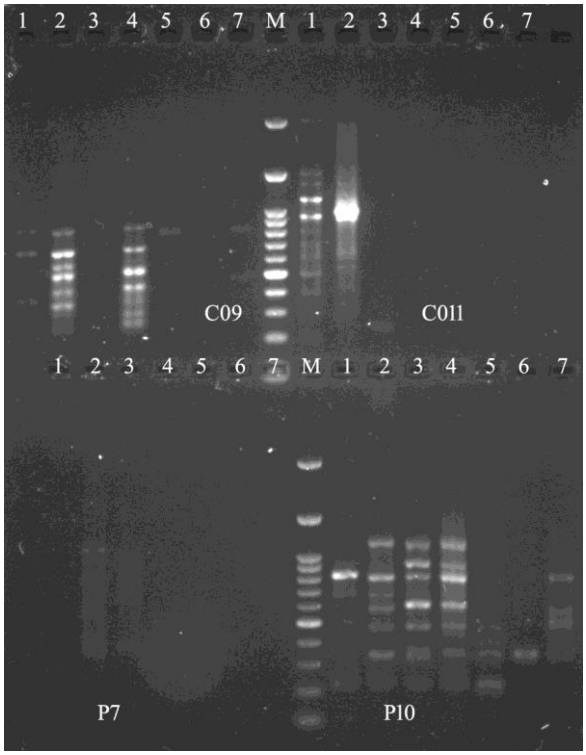

Figure 2. Gel electrophoresis for four RAPD primers of seven common carps.

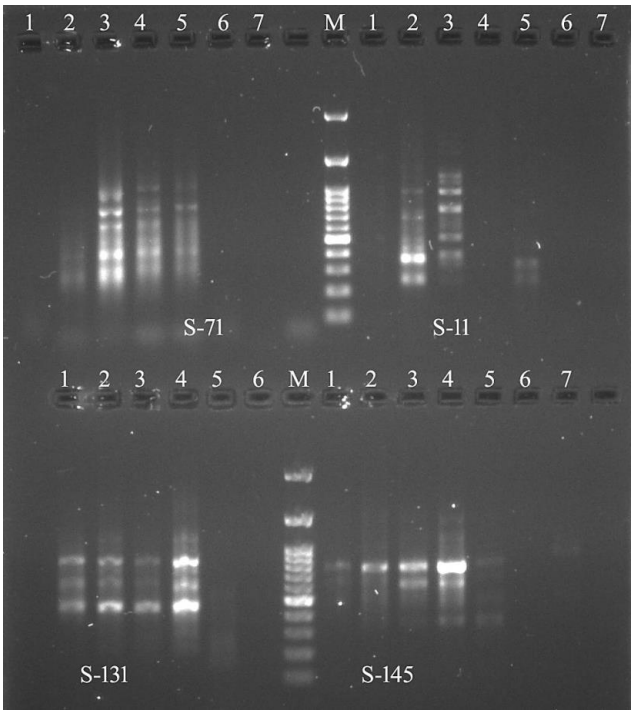

Fig. 3. Gel electrophoresis for four RAPD primers of seven common carps.

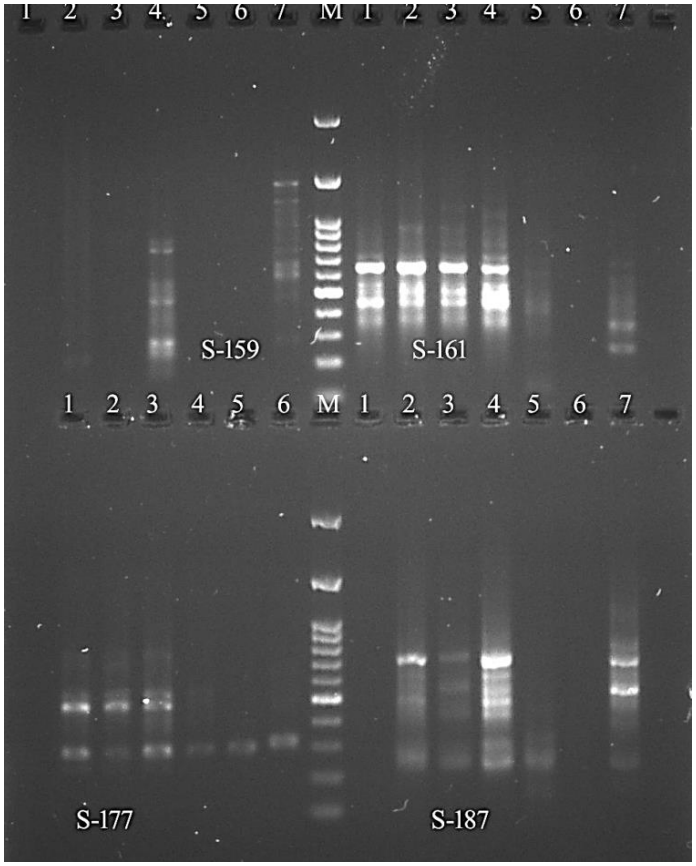

Figure 4. Gel electrophoresis for four RAPD primers of seven common carps.

Table 2. Band numbers and fragments size range (bp) in common carps.

Primer name	Fish samples													
	1		2		3		4		5		6		7	
	No. of band	Size range bp	No. of band	Size range bp	No. of band	Size range bp	No. of band	Size range bp	No. of band	Size range bp	No. of band	Size range bp	No. of band	Size range bp
C04	-	-	2	150-850	-	-	-	-	-	-	-	-	-	-
C06	-	-	-	-	-	-	-	-	3	650-980	-	-	-	-
C07	-	-	4	500-2000	-	-	-	-	-	-	-	-	-	-
C08	-	-	3	700-1200	1	700	1	700	-	-	-	-	-	
C09	3	350-750	6	230-750	-	-	7	250-850	1	750	-	-	1	750
C011	5	200-3000	3	550-950	1	250	-	-	-	-	-	-	-	-
P7	-	-	5	350-1100	3	380-1000	-	-	-	-	-	-	-	-
P10	3	220-950	7	220-1250	8	220-1250	8	220-1250	4	220-500	1	350	5	500-1100
S-71	-	-	2	300-450	5	300-950	5	300-1000	5	300-1000	-	-	-	-
S-11	-	-	4	250-950	6	250-1250	-	-	2	250-310	-	-	-	-
S-131	3	490-850	4	490-1250	3	490-850	4	490-850	-	-	-	-	-	-
S-145	2	700-850	1	850	3	390-850	6	390-1500	2	390-850	-	-	1	1000
S-159	-	-	1	200	-	-	6	200-800	-	-	-	-	7	290-1500
S-161	4	350-650	5	350-950	5	350-1200	7	350-1200	3	120-640	-	-	4	250-700
S-177	4	270-750	4	250-750	4	250-750	1	290	1	300	1	310	-	-
S-187	-	-	3	250-750	4	250-750	7	250-750	2	250-300	-	-	5	250-800
Total	24	200-3000	54	150-2000	43	220-1250	52	200-1500	23	120-1000	2	310-350	23	250-1500

Table 3. Polymorphic fragment numbers and polymorphic fragment percentages of sixteen primers.

Primer name	Total fragment number	No. of polymorphic fragments	Polymorphic fragments (%)
C04	2	2	100
C06	3	3	100
C07	4	4	100
C08	5	3	60
C09	17	11	64.7
C011	9	8	88.88
P7	8	5	62.5
P10	36	12	33.33
S-71	18	6	33.33
S-11	12	9	75
S-131	14	4	28.57
S-145	15	7	46.66
S-159	14	11	78.57
S-161	28	14	50
S-177	15	8	53.33
S-187	21	9	42.85
Total	221	116	52.48

Table 4. Unique band numbers and fragments size in common carps.

Primer name	Fish samples													
	1		2		3		4		5		6		7	
	No. of band	Size range bp	No. of band	Size range bp	No. of band	Size range bp	No. of band	Size range bp	No. of band	Size range bp	No. of band	Size range bp	No. of band	Size range bp
C04	-	-	2	150-850	-	-	-	-	-	-	-	-	-	-
C06	-	-	-	-	-	-	-	-	3	650-980	-	-	-	-
C07	-	-	4	150-2000	-	-	-	-	-	-	-	-	-	-
C08	-	-	2	850-1250	-	-	-	-	-	-	-	-	-	
C09	-	-	3	230-570	-	-	5	250-850	-	-	-	-	-	-
C011	4	1250-3000	2	550-950	1	250	-	-	-	-	-	-	-	-
P7	-	-	3	350-1100	1	1000	-	-	-	-	-	-	-	-
P10	-	-	-	-	-	-	-	-	2	220-400	-	-	3	510-800
S-71	-	-	-	-	1	1000	-	-	-	-	-	-	-	-
S-11	-	-	1	600	4	500-1250	-	-	1	310	-	-	-	-
S-131	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S-145	-	-	-	-	-	-	3	500-1500	-	-	-	-	1	1000
S-159	-	-	-	-	-	-	3	250-800	-	-	-	-	5	290-1500
S-161	-	-	-	-	-	-	1	700	-	-	-	-	2	250-340
S-177	-	-	-	-	-	-	-	-	1	300	-	-	-	-
S-187	-	-	-	-	-	-	1	250-750	-	-	-	-	2	550-800

Table 5. Estimation of heterozygosity and Shannon' index of sixteen primers.

Locus	h*	I*
C04	0.4898	0.6829
C06	0.4082	0.5983
C07	0.4082	0.5983
C08	0.2449	0.4101
C09	0.2449	0.4101
C011	0.2449	0.4101
P7	0.4082	0.5983
P10	0.4082	0.5983
S-71	0.2449	0.4101
S-11	0.2449	0.4101
S-131	0.2449	0.4101
S-145	0.2449	0.4101
S-159	0.2449	0.4101
S-161	0.2449	0.4101
S-177	0.4082	0.5983
S-187	0.4082	0.5983
Mean	0.3214	0.4977

* h = Nei's (1973) gene diversity.
* I = Shannon's Information index.

Genetic distance

The Nei's genetic distances among seven fish samples in this study was ranged from 0.1335 to 0.9808 (Table 6). The lowest genetic distance recorded between types 3 and 4 on one side and 4 and 6 on the other side was 0.1335, and the highest genetic distance recorded among types 1 and 5 was 0.9808 (Table 6). These results showed the effect of lakes (Dukan and Darbandikhan) on genetic diversity among common carps.

Phylogenetic tree construction

The dendrogram (Fig. 5) showed six clusters; the 1st cluster branch consisted of the type 3 and 4, the 2nd cluster was including type 6, the 3rd cluster including only type 2, the 4th cluster including type 1, the 5th cluster including type 7 and the 6th cluster including type 5. These results indicated that the type 1 is most genetically distant from the type 5 (0.9808). The type 3 and 4 in the 1st cluster indicating a close relationship between them and the results indicate that the fishes in types 3 and 4 were closer to type 6. The dendrograms showed that there is a genetic diversity among the seven fish samples ranged from 0.1335 to 0.9808.

Table 6. Genetic distance among the seven common carps.

Fish samples	1	2	3	4	5	6	7
1	****						
2	0.3747	****					
3	0.5754	0.2877	****				
4	0.3747	0.2877	0.1335	****			
5	0.9808	0.8267	0.5754	0.3747	****		
6	0.3747	0.4700	0.2877	0.1335	0.5754	****	
7	0.5000	0.4700	0.2877	0.4700	0.8267	0.6931	****

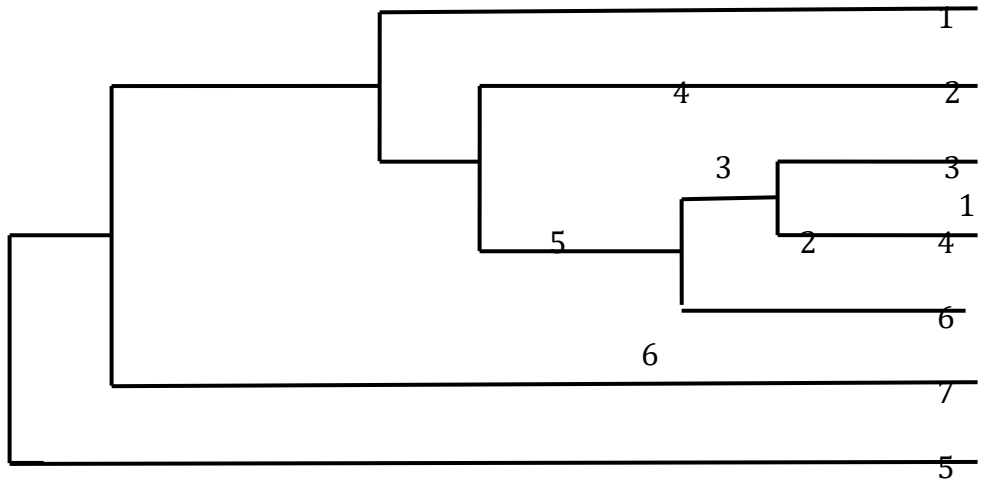

Figure 5. UPGMA dendrogram showing differentiation between the seven common carps based on Nei (1972) genetic distance.

DISCUSSION

C. carpio, as a genetically diverse and successful species, has been adapted to various environments across a broad ecological spectrum in Eurasia and has been domesticated for more than 2,000 years. This species has been bred into numerous strains and local populations, producing distinct phenotypic changes in its growth rate, temperature and hypoxia tolerance, body color, scale pattern and body shape, which are partially attributable to genome diversity due to its two WGD (3R and 4R) events (Xu et al., 2014).

The 223 fragments from 16 primers found in this study were higher than the result reported by Ramanadevi et al. (2013) in two Indian mudskipper species *Boleophthalmus boddarti* and *B. dussumieri* (TFN was 104), Petjul et al. (2011) in *Barbodes* spp. TFN arrived. On the other hand, the present results of fish diversity is lower than that reported by Faddagh et al. (2012) where TFN was 223, Wali et al. (2013) in *C. carpio var. communis, C. carpio var. specularis* and *Carassius carassius* (TFN was 3371).

The results in table (3) indicated that it is possible to depend upon these loci for genetic diversity among fish samples. The PFN in this study was lower than that reported by Wali et al. (2013) where PFN reached 3008. The result in table (5) indicated that the genetic diversity between fish samples is moderately high. The gene diversity value in this study was higher than those reported by Ramanadevi et al. (2013) for *B. boddarti* and *B. dussumieri* which were 0.0116 ± 0.0066 and 0.0056 ± 0.0024, respectively.

The genetic distances among fish samples in the present study were higher than that reported by Faddagh et al. (2012) in eight cyprinid fish species. However, genetic distance ranged from 7.6% to 98.1%.

CONCLUSION

The high genetic diversity among the seven samples of common carps in this study explains that there are seven common carps which are different from each other. These results showed the effects of geographical location of lakes and distance on the genetic diversity of common carps.

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