Genetic Differences within and between Populations of Korean Catfish (*S. asotus*) and Bullhead (*P. fulvidraco*) Analysed by RAPD-PCR

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ABSTRACT : Of the 20 arbitrarily chosen primers, six oligonucleotides decamer primers were used on the basis of the number of the polymorphisms generated in catfish (*Silurus asotus*) from Yesan and bullhead (*Pseudobagrus fulvidraco*) from Dangjin in Korea. Six primers were used generating a total of 602 scorable bands in catfish and 195 in bullhead population, respectively, ranging in size of DNA fragments from less than approximately 100 to larger than 2,000 base pairs (bp). Six primers yielded 199 polymorphic fragments (33.1%) in catfish and 47 (24%) in bullhead, respectively. In the present study, a total of 328 common fragments (an average of 54.7 per primer) were observed in catfish population, whereas 84 (an average of 14.0 per primer) in bullhead. The total number of specific fragments in catfish and bullhead population were 76 and 64, respectively. In catfish population, random decamer, OPA-17 (GACCGCTTGT) generated the highest number of fragments (a total of 141) in comparison with other primers used, with an average of 11.8. The common bands in the molecular weight of 300 bp generated by random primer OPA-06 (GGTCCCTGAC) were present in every individuals in bullhead population. The major polymorphic bands in the molecular weight of 100 bp generated by OPA-17 were identified in lane 14, 15, 17, 18, 19 20 and 21, which were identifying species in bullhead population. The average bandsharing values (BS values) of all of the samples within catfish population ranged from 0.575 to 0.945, whereas 0.063-1.000 within bullhead population. The bandsharing value (index of similarity between individuals) between individual No. 5 and No. 9 showed the highest level within catfish population, whereas the bandsharing value between individual No. 1 and No. 2 showed the lowest level. The single linkage cluster analysis resulted from four primers, indicating four genetic groupings composed of group 1 (C1-C10, all of the catfish samples), group 2 (B11, B12, B13, B14, B16, B17, B18, B19), group 3 (B15) and group 4 (B20 and B21). The dendrogram reveals close relationships between individual identities within two species populations and individuals derived from the same ancestor, respectively. However, genetic distances between two species populations ranged from 0.124 to 0.333. The shortest genetic distance (0.042) displaying significant molecular differences was between individual No. 6 and No. 9 catfish population. The shortest genetic distance (0.033) displaying significant molecular differences also was between individual No. 18 and No. 19 in bullhead population. Reversely, the genetic distance of individual No. 20/21 among individuals in bullhead population was highest (0.333). This result showed that bullhead No. 20 and 21 were distinct from other individuals within bullhead population. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 8 : 1053-1061)***

Key Words : Bullhead, Catfish, Dendrogram, Genetic Distance, *Pseudobagrus fulvidraco*, Random Primer, *Silurus asotus*, Similarity Index

INTRODUCTION

Peptide, allozyme and mitochondrial or genomic DNA molecular markers by polyacrylamide and agarose gel electrophoresis (PAGE and AGE) have had many feasible applications in animal, plant and microbe biological inquiry. There were so far used various PCR-based molecular biological methods including restriction fragment length polymorphisms (RFLPs) (Bommineni et al., 1997), amplified fragment length polymorphisms (AFLPs) (Pejic et al., 1994), random amplified polymorphic DNAs (RAPD) (Johnson et al., 1994; Partis and Wells, 1996; Iyengar et al., 2000; Mohd-Azmi et al., 2000; Yoon, 2001; Appannavar et al., 2003), microsatellite (Huang et al., 2000; Iyengar et al., 2000) and DNA sequencing (Debenham et al., 2000). Especially, the polymorphic/specific markers unique to species, breed and population have been used for individual or species discrimination, population genetics, identification of traits like disease resistance and for breed improvement in genetics and breeding programs (Tassanakajon et al., 1998; McCormack et al., 2000; Klinbunga et al., 2000a, 2000b; Yoon, 2001; Ramesha et al., 2002).

Due to the variation in RAPD profiles and difficulties with reproducibility, the scoring of RAPD fragments was done very conservatively (Moeller and Schaal, 1999). However, many genetic researches were made because RAPD-PCR is a rapid and good method for identifying genetic diversity and similarity between various life organisms with the advantage that no prior knowledge of the genome under research is needed (Welsh et al., 1991; Spooner et al., 1997; Pejic et al., 1998; Fischer et al., 2000;

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Nebauer et al., 2000; Geng et al., 2002). Also, genomic characterization generated by PCR amplification of DNA using random primers has compatibility for detecting DNA polymorphisms among various life organisms (Welsh et al., 1991; Dias Neto et al., 1993; Orozco-Castillo et al., 1994; Vierling et al., 1994). Especially, identification of the genetic distances between breeds or populations is necessary for effective conservation and management of population, species, breeds and lines (Klinbunga et al., 2000b; Appannavar et al., 2003).

Catfish (*S. asotus*) and bullhead (*P. fulvidraco*), economically important aquacultural species, belonging to order Siluriformes including two family Siluridae and Bagridae, respectively, are considered to be closely related to each other by various authors (Kim, 1997). Two fish species widely inhabit the slow river and stream that flows to the West Sea and South Sea in the Korean Peninsula. Especially, bullhead is widely distributed in the river and stream in North Korea, Japan China and Siberia. Catfish and bullhead are one of the most intensively studied and reared fish species for real income enhancement of farmers because of their importance as a food and sport fish. In spite of its economic and scientific consequence, little information is available on the genetic relationships between two species in Korea. Thus, the applications of RAPD to aquaculture had been to identify genetic similarity and differences between a few of fish species and/or mollusks apart from geographic sites (Callejas and Ochando, 1998; Tassanakajon et al., 1998; Hamm and Burton, 2000; Yoon and Park, 2001). Especially, neighbor-joining tree analysis of the genetic distance among fish species or populations using RAPD-PCR is of little quantity (Tassanakajon et al., 1998; Klinbunga et al., 2000b; McCormack et al., 2000).

There is a need to understand the genetic characteristics and composition of two fish species in order to evaluate exactly the latent genetic effects induced by seed production operations. This research was made by RAPD-PCR using two decades of random primers and also by single linkage cluster analysis in order to elucidate genetic differences within and between populations in Korean catfish (*S. asotus*) from Yesan and bullhead (*P. fulvidraco*) from Dangjin neighboring Yesan and also to identify suitable RAPD markers peculiar to these species.

MATERIALS AND METHODS

Blood collection and sources of genomic DNA

Blood samples were obtained from Korean catfish (*S. asotus*) from Yesan and bullhead (*P. fulvidraco*) from Dangjin adjacent to Yesan, respectively. RAPD-PCR analysis was performed on DNA samples from a total of 22 individuals using six of decamer primers of two decades of different random primers.

In order to achieve good and reproducible results, DNA extraction should be carried out according to the following separation and extraction methods.

Samples of blood were placed into 10 ml test tubes, to which an 4 volumes of lysis buffer I (155 mM NH_4Cl, 10 mM $KHCO_3$, 1 mM EDTA) was added, and the mixture tubes were gently inverted several times. The samples were incubated on ice for 30 min, centrifuged at 1,750 g for 10 min at 4°C to pellet. The supernatants were decanted and the pellets were resuspended with lysis buffer I, and then mixtures were centrifuged with microcentrifuge at 1,750 g for 10 min at 4°C. The precipitates were diffused with lysis buffer II (10 mM Tris-HCl, pH 8.0, 10 mM EDTA, 100 mM NaCl, 0.5% SDS). Samples were added 15 μl proteinase K solution (10 mg/ml).

The mixtures were gently inverted and incubated at 65°C for overnight. After incubation, suspended solutions (samples) were transferred to 1.5 ml Eppendorf tubes. There was added 300 μl of 6 M NaCl and gently pipetted for a few of min. 600 μl of chloroform were added to the mixture and then inverted (no phenol). Samples were spun down at 22,388 g for 5 min. The cleared lysates were extracted with 2 volume of ice-cold 70% ethanol, then centrifuged at 6,289 g for 5 min, then precipitated. The DNA pellets were incubation-dried for 3 h, and then dissolved in the pure distilled water. Purity and final concentration of DNA was estimated by calculating the ratios of the absorbance measured at 260 nm with a spectrophotometer (Shimadzu, Australia).

Primer, marker and amplification conditions

Two decades of decamer primers (5' to 3') obtained from Operon Technologies, USA had a G+C content in the range 60-70%. Of the 20 arbitrary primers, six random primers showing reproducible and clearly scorable, polymorphic and specific fragments, were used to identify genetic similarity and difference. RAPD-PCR was performed using a DNA Thermal Cycler (Perkin Elmer Cetus, USA). RAPD amplification reactions were undertaken in volumes of 20 μl contained 10 ng of template DNA, 20 μl premix (Super-Bio Co., Korea) and 1.0 unit primer. This mixture was followed a pre-denaturation at 94°C for 5 min. The thermal cycler programmed for 45 cycles at 94°C for 1 min for denaturation, at 36°C for 1 min for annealing, at 72°C for 1 min for extension, at 72°C for 5 min for post-extension, using the fastest available transition between each temperature. Amplification products were separated by electrophoresis with ΦX174 DNA/*Hae*III marker (Promega Co., USA) in 1.4% agarose gels with TBE (90 mM Tris, pH 8.5; 90 mM borate; 2.5 mM EDTA) and detected by staining with ethidium bromide (EtBr). The

Table 1. Average length of RAPD-PCR markers on catfish (*S. asotus*) from Yesan and bullhead (*P. fulvidraco*) from Dangjin.

Primer	Species	Average length of RAPD-PCR fragments													
		A	B	C	D	E	F	G	H	I	L	M	N	O	P
OPA-O6	Catfish	1,100	900	700	600	500	450	400	350	310	300	280	250		
	bullhead	700	600	450	400	300	250								
OPA-O9	Catfish	900	800	700	600	550	500	480	450	400	350	320	300	280	
	bullhead	700	600	450	350	280									
OPA-13	Catfish	800	700	650	600	500	400	350	310	280	230	200			
	bullhead	1,400	700	650	500	450	400	300	250	200	150				
OPA-14	Catfish	1,300	1,000	900	800	700	600	500	400	350	300	250	200	150	
	bullhead	400	300	150											
OPA-17	Catfish	2,000	1,400	1,000	900	800	750	700	600	500	400	350	300	250	100
	bullhead	550	500	400	300	280	220	150	100						
OPA-19	Catfish	1,100	900	800	650	550	500	400	380	300	280	250	200		
	bullhead	550	250	150											

Table 2. Number of total, average, common, specific and polymorphic bands generated from RAPD patterns for 6 arbitrary primers in catfish (10-12 fishes/primer) and bullhead (8-11 fishes/primer)

Primer	No. of average band per lane		No. of common bands		No. of specific bands		No. of polymorphic bands	
	Catfish	Bullhead	Catfish	Bullhead	Catfish	Bullhead	Catfish	Bullhead
OPA-06	7.3 (73)	3.5 (38)	30	11	12	11	31	16
OPA-09	3.5(132)	2.8 (28)	36	10	14	18	34	0
OPA-13	8.5 (85)	5.6 (62)	66	33	26	10	0	19
OPA-14	10.5(116)	1.6 (13)	66	8	15	5	35	0
OPA-17	10.9(109)	3.2 (35)	90	11	3	17	29	7
OPA-19	8.7 (87)	1.7 (19)	40	11	6	3	70	5
Total no.	49.4(602)	18.4(195)	328	84	76	64	199	47
Average no. per primer	8.2	3.1	54.7	14.0	12.7	10.7	33.2	7.8

Parentheses are the total number of products generated in catfish and bullhead.

agarose gels electrophoresed were illuminated with UV light and taken photographs by photoman direct copy system (Seoulin Co., Korea).

Analytical method

All reproducible bands ranging from less than approximately 100 to larger than 2,000 bp were scored from the gel. Several primers that revealed minor bands and no polymorphisms, were excluded from the analysis. Only bands that were readily visible were scored. Bandsharing values was calculated using the formula of Nei and Li (1979), Jeffreys and Morton (1987) and Mohd-Azmi et al. (2000) to measure the genetic similarity of RAPD fingerprints: BS=N (Na.....n)/(Na+Nb+.....+Nn). Where Nabc is the number of bands shared by individuals a, b and c, Na is the total number of bands for individual a, Nb is the total number of bands for individual b and Nc is the total number of bands for individual c. For instance, as follows: BS=2 (Nab)/(Na+Nb). Where Nab is the number of bands shared by individuals a and b, Na is the total number of bands for individual a, and Nb is the total number of bands for individual b. An average of within-population similarity is calculated across all pairwise comparisons between individuals within a population. Single linkage cluster analysis was performed on the similarity matrices in order to generate a dendrogram using pc-package program Systat version 10 (SPSS Inc., USA). Genetic distances within and between populations were calculated with dendrograms produced with Systat version 10. BS values were scored by the presence or absence of an amplified product at specific positions in the same gel from the RAPD profiles. PCR amplification and bandsharing analyses on the same DNA sample were carried out to examine the efficiency and then the data obtained were used in this experiment and data analyses above-mentioned.

RESULTS

RAPD-PCR variation

Of the 20 arbitrarily chosen primers, six oligonucleotides decamer primers were used on the basis of the number of the polymorphisms generated in Korean catfish (*S. asotus*) from Yesan and bullhead (*P. fulvidraco*) from Dangjin. The random primers OPA-06, -09, -13, -14, -17 and -19 showed polymorphic bands generated using each primer to amplify DNA isolated from the blood of individuals (Figures 2A, -B, -C, and -D) (Tables 1-3). Six primers were used generating a total of 602 scorable bands in catfish and 195 in bullhead population, respectively, ranging in size of DNA fragments from less than approximately 100 to larger than 2,000 base pairs (bp) (Tables 1 and 2). 6 random primers yielded amplified

Table 3. Similarity matrix calculated using Nei and Li's index of similarity for catfish and bullhead

		Bandsharing values of catfish										Bandsharing values of bullhead										
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Genetic	1	-	0.575	0.616	0.785	0.734	0.704	0.718	0.817	0.855	0.817	0.431	0.321	0.404	0.258	0.389	0.556	0.558	0.563	0.446	0.091	0.091
differences	2	0.425	-	0.895	0.769	0.858	0.797	0.717	0.603	0.760	0.768	0.561	0.425	0.502	0.374	0.325	0.516	0.381	0.531	0.437	0.178	0.178
of catfish	3	0.385	0.105	-	0.753	0.841	0.771	0.755	0.638	0.750	0.895	0.522	0.613	0.522	0.514	0.309	0.488	0.600	0.636	0.617	0.202	0.202
	4	0.216	0.232	0.247	-	0.791	0.764	0.777	0.797	0.771	0.877	0.439	0.475	0.293	0.335	0.309	0.450	0.358	0.420	0.408	0.206	0.206
	5	0.267	0.142	0.159	0.225	-	0.843	0.859	0.774	0.945	0.886	0.398	0.398	0.321	0.294	0.318	0.450	0.377	0.517	0.496	0.174	0.174
	6	0.296	0.204	0.230	0.239	0.157	-	0.860	0.777	0.720	0.889	0.381	0.381	0.304	0.287	0.206	0.328	0.357	0.693	0.405	0.167	0.167
	7	0.282	0.284	0.246	0.229	0.141	0.140	-	0.792	0.866	0.904	0.389	0.543	0.398	0.367	0.311	0.438	0.450	0.580	0.508	0.170	0.170
	8	0.184	0.398	0.453	0.203	0.230	0.229	0.214	-	0.678	0.791	0.367	0.367	0.267	0.263	0.261	0.393	0.322	0.486	0.393	0.063	0.063
	9	0.146	0.241	0.255	0.234	0.056	0.280	0.134	0.322	-	0.929	0.365	0.365	0.288	0.308	0.172	0.336	0.354	0.586	0.473	0.188	0.188
	10	0.182	0.232	0.104	0.123	0.114	0.111	0.134	0.210	0.072	-	0.408	0.408	0.408	0.374	0.325	0.463	0.472	0.531	0.508	0.178	0.178
Genetic	11	0.569	0.439	0.494	0.561	0.603	0.619	0.616	0.634	0.636	0.593	-	0.611	0.611	0.650	0.486	0.536	0.619	0.622	0.649	0.417	0.417
differences	12	0.680	0.580	0.393	0.526	0.618	0.619	0.463	0.634	0.636	0.593	0.434	-	0.900	0.650	0.686	0.536	0.873	0.734	0.788	0.417	0.417
of bullhead	13	0.596	0.478	0.484	0.707	0.680	0.696	0.616	0.734	0.713	0.593	0.434	0.100	-	0.400	0.686	0.536	0.762	0.834	0.883	0.417	0.417
	14	0.743	0.626	0.487	0.665	0.707	0.714	0.634	0.738	0.692	0.636	0.350	0.350	0.600	-	0.619	0.452	0.645	0.643	0.655	0.667	0.667
	15	0.612	0.675	0.639	0.691	0.683	0.795	0.689	0.740	0.829	0.675	0.515	0.315	0.315	0.381	-	0.786	0.667	0.536	0.584	0.667	0.667
	16	0.445	0.484	0.512	0.550	0.550	0.673	0.562	0.607	0.665	0.537	0.465	0.465	0.465	0.548	0.215	-	0.667	0.740	0.695	0.500	0.500
	17	0.442	0.619	0.400	0.647	0.623	0.643	0.550	0.679	0.646	0.528	0.381	0.127	0.238	0.356	0.333	0.333	-	0.845	0.775	0.400	0.400
	18	0.302	0.469	0.364	0.580	0.534	0.308	0.420	0.515	0.415	0.469	0.373	0.267	0.167	0.357	0.500	0.500	0.500	-	0.855	0.310	0.310
	19	0.555	0.564	0.384	0.597	0.504	0.595	0.493	0.608	0.527	0.493	0.361	0.212	0.117	0.346	0.417	0.306	0.225	0.146	-	0.343	0.343
	20	0.909	0.823	0.798	0.795	0.827	0.834	0.830	0.938	0.812	0.823	0.584	0.584	0.584	0.334	0.333	0.500	0.600	0.691	0.657	-	1.000
	21	0.909	0.821	0.798	0.795	0.827	0.834	0.830	0.938	0.812	0.823	0.584	0.584	0.584	0.334	0.500	0.500	0.600	0.691	0.657	0.000	-

fragments that were consistently polymorphic and specific between two populations.

In catfish population, this population variation was revealed in the band patterns ranged from approximately 250 to 1,100 base pairs generated by random primer OPA-06 (GGTCCCTGAC) (Figure 2A). The identical band patterns were observed in approximately 500 and 600 bp, respectively. The common band patterns were also observed in approximately 450 bp. The specific major and/or minor bands in the molecular weight in approximately 280, 350 and 400 bp, respectively, were observed in lane 2, 3, 4, 5, 6, 7, 9 and 10. The common band patterns by random primer OPA-13 (CAGCACCCAC) were observed from approximately 280 to 800 bp (Figure 2B). The common major band pattern in the molecular weight of 300 bp and 500 bp was observed in every lane. Also, the identical minor band generated by this primer was observed from 310 to 500 bp. Especially, the specific band in the molecular weight of approximately 250 bp was observed in lane 5, 6, 8, 9 and 10. Also, the specific band pattern was observed in lane 3, 4, 6, 7, 10 and 11 (molecular weight, larger than 600 bp). Random decamer, OPA-17 (GACCGCTTGT), detected common major bands in molecular weight from 250 to 1,000 bp, which were identical (Figure 2C). The specific major bands in approximately 1,400 and 1,600 bp were observed in lane 4, 11 and 12, respectively. Especially, the common bands in molecular weight 2,000 bp were present in every individual except for lane 1. Especially, this primer produced the highest number of fragments (a total of 141) in comparison with other primers used, with an average of 11.8. The common major band patterns generated by random primer OPA-19 (CAAACGTCGG) were observed in approximately 310 bp, 400 bp, 600 bp and 800 bp, respectively (Figure 2D). The polymorphic bands ranged from 200 to 700 bp generated by this primer exhibited the inter-individual-specific characteristics and showed DNA polymorphisms. Also, the specific bands in the molecular weight of approximately 350 and 1,100 bp were observed in lane 1, 2, 3 and 10, respectively.

In bullhead population, the RAPD-PCR variation was revealed in the band patterns ranged from approximately 250 to 700 bp generated by random primer OPA-06 (Figure 2A). The common bands in the molecular weight of 300 bp were present in every individual. The amplified DNA fragment of approximately 450 bp was specific to three individuals (lane 14, 18 and 19). The specific band patterns of RAPD products were observed in lane 13 and 18 (approximately 700 bp). The identical fragments from 300 to 400 bp generated by random primer OPA-13 were analyzed (Figure 2B). Especially, this primer produced the highest number of fragments (a total of 62) in comparison with other primers used, with an average of 5.6. This primer generated specific RAPD profiles of major DNA band of 1,400 bp in lane 12. The specific RAPD fragments also were observed in lane 12 (molecular weight, 700 bp) and lane 18 (approximately 500 bp), respectively. The common band patterns generated by random primer OPA-17 were observed in molecular weight size of 300 bp (Figure. 2C). The specific fragments recorded in 550 bp (lane 18, 20 and 21), 400 bp (lane 13, 17, 18 and 20) and 200 bp (lane 13), respectively, in the molecular weight produced by this random primer were observed. The major polymorphic

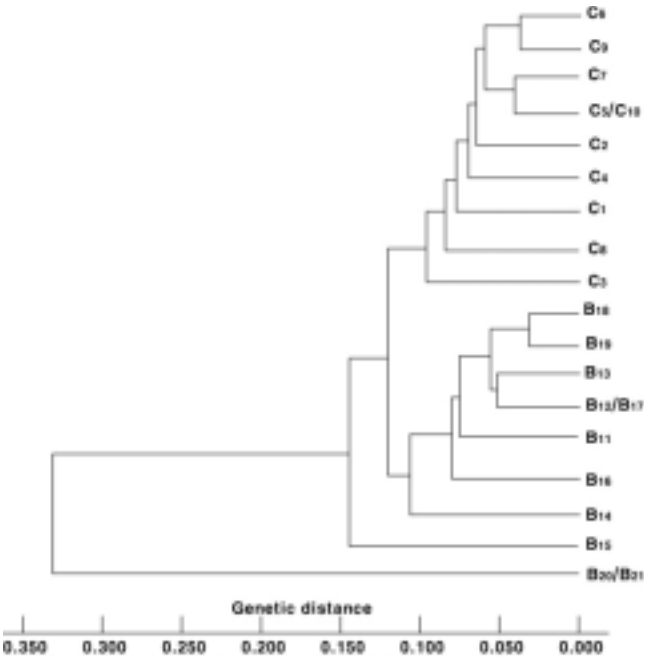

Figure 1. Dendrogram of genetic distances showing the relatedness among different individuals of catfish (C1-C10) and bullhead (B11-B21) generated according to the bandsharing values and genetic differences matrix in Table 3.

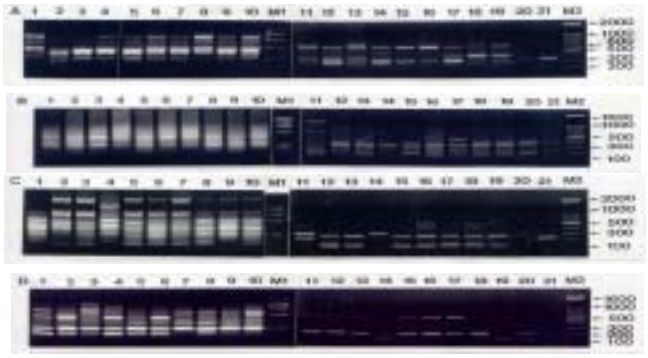

Figure 2. Amplification products were electrophoresed on a 1.4% agarose gel with TBE (90 mM Tris, pH 8.5; 90 mM boric acid; 2.5 mM EDTA) and detected by staining with ethidium bromide. Individual specific RAPD patterns of catfish (lane 1-10) and bullhead (lane 11-21) amplified by arbitrary primer OPA-06 (A), OPA-13 (B), OPA-17 (C) and OPA-19 (D), respectively. Each lane shows different individual DNA samples. Molecular size marker: M1, ΦX174 DNA marker digested with *Hae*Ⅲ; M2, 100 bp DNA ladder.

bands in the molecular weight of 100 bp generated by this primer were identified in lane 14, 15, 17, 18, 19 20 and 21, which were identifying species. The decamer primer, OPA-19, detected common fragments in molecular weight size of 200 bp, that were identical (Figure 2D). The specific RAPD fragments in the sizes of molecular weight of approximately 150 bp were observed in lane 15, 20 and lane 21, respectively. The polymorphic fragments in the molecular weight of 500 bp generated by this primer were identified in lane 12, 13, 14, 15, 16, 17 and 18, which were identifying species.

Within- and between-populations variations and bandsharing values

In the present study, six primers generated a total of 602 fragment in catfish and 195 in bullhead, of which 199 polymorphic fragments (33.1%) in catfish and 47 (24%) in bullhead, respectively (Table 2). A total of 328 common fragments (an average of 54.7 per primer) were observed in catfish, whereas 84 (an average of 14.0 per primer) in bullhead. The total number of specific fragments in catfish population and bullhead population were 76 and 64, respectively. The similarity indices based on Nei and Li's index are reported (Table 3). The average bandsharing values of all of the samples within catfish population ranged from 0.575 to 0.945, whereas 0.063-1.000 within bullhead population. The bandsharing value between catfish No. 5 and No. 9 showed the highest level within catfish population, whereas the bandsharing value between catfish No. 1 and No. 2 showed the lowest level. Also, the bandsharing value between bullhead No. 20 and No. 21 showed the highest level within catfish population, whereas the bandsharing value between bullhead No. 8 and No. 21 showed the lowest level. A similarity matrix based on Nei and Li's index of similarity was used to perform single linkage cluster analysis in order to obtain the dendrogram (Figure 1). The single linkage dendrogram resulted from four primers, indicating four genetic groupings composed of group 1 (C1-C10, all of the catfish samples), group 2 (B11, B12, B13, B14, B16, B17, B18, B19), group 3 (B15) and group 4 (B20 and B21). The dendrogram reveals close relationships between individual identities within two species populations and individuals derived from the same ancestor. However, genetic distances between two species populations ranged from 0.124 to 0.333. The shortest genetic distance (0.042) displaying significant molecular differences was between catfish No. 6 and No. 9. The shortest genetic distance (0.033) displaying significant molecular differences also was between bullhead No. 18 and No. 19. Especially, the genetic distance of bullhead No. 20/21 among individuals in two species was highest (0.333). This result showed that bullhead No. 20 and 21 were distinct from other individuals within bullhead population.

The oligonucleotide primer OPA-13 produced identical DNA fragments whose sizes of approximately 310 and 400 bp, respectively, between catfish population and bullhead (Figure 2B). As calculated by bandsharing analysis, the bandsharing values varied from 0.308 to 1.000 in catfish population and also from 0.000 to 1.000 in bullhead population, respectively (Table 3). The average level of bandsharing value (mean±SD) was approximately 0.661±0.123 in catfish population and 0.504±0.115 in

bullhead population, respectively. BS value in catfish population was higher than that in bullhead population.

DISCUSSION

RAPD-PCR variation

In the present study, six primers were used generating a total of 602 scorable bands in catfish and 195 in bullhead population, respectively, ranging in size of DNA fragments from less than approximately 100 to larger than 2,000 bp, as summarized in Table 1 and 2. Of the 30 RAPD primers used, 25 detected polymorphism with an average of three polymorphic loci per primer in coffee (Orozco-Castillo et al., 1994). A total of 80 bands ranging from 200 to 2,200 bp in size were unambiguously scored in black tiger shrimp (Tassanakajon et al., 1998). Amplified fragments obtained by RAPD ranged in size from 220 to 3,000 bp for all primers in native American maize accessions (Moeller and Schaal, 1999). The number of amplified bands across all investigated samples was 36, 32 and 24 bands for primers, respectively (Klinbunga et al., 2000b). DNA fragments obtained by four primers were ranged in size from 100 to 2,300 bp in brittle star (McCormack et al., 2000). A total of 73.7 average number of polymorphic products were observed between common carp and Israeli carp (Yoon, 2001). On average, each random RAPD primer generated 8.2 amplified products. Each random RAPD primer produced an average of amplified 10 fragments from 7.7 to 13.5 bands. Also, five primers were used generating a total of 1,084 scorable bands in wild and cultured populations of crucian carp, ranging in size from 120 to larger than 4,270 bp (Yoon and Park, 2001). Geng et al. (2002) reported that the length of these DNA fragments analysed by 8 random primers were 176-2,937 bp.

In catfish population, this population variation was revealed in the band patterns ranged from approximately 250 to 1,100 base pairs generated by random primer OPA-06, as shown in Figure 2A. The identical band patterns were observed in approximately 500 and 600 bp, respectively. The common band patterns were also observed in approximately 450 bp. The common major band patterns generated by random primer OPA-19 were observed in approximately 310 bp, 400 bp, 600 bp and 800 bp, respectively, as shown in Figure 2D. The polymorphic bands ranged from 200 to 700 bp generated by this primer exhibited the inter-individual-specific characteristics and showed DNA polymorphisms. Also, the specific bands in the molecular weight of approximately 350 bp and 1,100 bp were observed in lane 1, 2, 3 and 10, respectively. The specific primer was found to be useful in the individual identification, resulting from the different DNA polymorphism among individuals (Liu et al., 1998). The RAPD-PCR method using arbitrary primers was applicable to identify three endemic Spanish barbel species (Callejas and Ochando, 1998). Also, there were population-related RAPD fragments in catfish and there were differences in frequencies of six primer fragments, as have been reported in catfish (Liu et al., 1998). In bullhead population, the RAPD-PCR variation was revealed in the band patterns ranged from approximately 250 to 700 bp generated by random primer OPA-06, as shown in Figure 2A. The common bands in the molecular weight of 300 bp were present in every individual. The amplified DNA fragment of approximately 450 bp was specific to three individuals (lane 14, 18 and 19). The specific band patterns of RAPD products were observed in lane 13 and 18 (approximately 700 bp). The identical fragments from 300 to 400 bp generated by random primer OPA-13 were analyzed, as shown in Figure 2B. These results indicated that the genome sizes of catfish and bullhead were similar to those of blue catfish analyzed by Liu et al. (1998). Out of RAPD markers obtained from 7 primers, 53 were common to all breeds, 22 were individual species and 18 were polymorphic for different breeds of zebu cattle (Ramesha et al., 2002). Generally, the size and number of the fragments generated unbiased depended on the nucleotide sequence of the primer used and on the source of the template DNA, resulting in a genome-specific DNA fragment.

Polymorphic bands, specific bands and bandsharing scores

The RAPD-PCR approach have been applied to identify the polymorphic/specific markers unique to species, breed and population and DNA polymorphisms in various animals/plants/microbes using a large number of random decamer primers (Spooner et al., 1997; Tassanakajon et al., 1998; Huang et al., 2000; Klinbunga et al., 2000a, 2000b; McCormack et al., 2000; Ramesha et al., 2002). In this study, six primers generated 199 polymorphic fragments (33.1% of a total of 602 fragment) in catfish and 47 (24% of 195 fragments) in bullhead, respectively, as summarized in Table 2. Overall, these results indicate a large number of polymorphic fragments detected per primer and suggest high genetic variation in catfish from Yesan and bullhead population from Dangjin. The percentages of polymorphic bands of the five geographic populations investigated in black tiger shrimp (*Penaeus monodon*) varied from 51.5 to 57.7% (Tassanakajon et al., 1998). They reported that RAPD analysis yielded a total of 252 genotypes. Two primers yielded the highest level of polymorphism, which was 88.9% in black tiger shrimp (Tassanakajon et al., 1998). Twenty-two of 80 bands (27.5%) were also monomorphic and 58 bands (72.5%) were polymorphic. Using RAPD analysis, they observed several species-specific bands in wild individuals of 3 commercially cultured oyster species, whereas didn't observe any population-specific markers

(Klinbunga et al., 2000a). Six primers produced 84 polymorphic bands out of a total of 90 bands in blacklip abalone (Huang et al., 2000).

Polymorphisms were scored by the presence or absence of the banding pattern of amplified fragments at specific positions expressed by various decamer primers (Johnson et al., 1994; Bommineni et al., 1997; Smith et al., 1997; Tassanakajon et al., 1998; Yoon and Park, 2001). The DNA fingerprint polymorphism and the genetic relationship were studied by RAPD technology on 3-goat populations from China Chaidamu Basin in China (Geng et al., 2002). They reported that the amplified bands were all 94 in 3 goat populations by using 8 random primers, and the DNA polymorphism frequencies of CG, CCG and LCG were 0.8404, 0.8617 and 0.8511, respectively. Six random primers produced from low to high numbers of polymorphic bands between pooled DNA of different Deoni types (Appannavar et al., 2003). Of the 48 RAPD markers obtained 33 were common to all Deoni types, 3 were individual specific and 12 were polymorphic for different Deoni types. They reported that the mean average percentage difference values among Deoni types showed that Balankya and Wannerra had less genetic divergence when compared Waghya. RAPD markers produced by primers were effective in determining polymorphism between sorghum lines (Vierling et al., 1994). The four primers used amplified a total of 91 DNA markers, ranging in size from 400 to 3,700 bp (Nebauer et al., 2000). The total number of amplified bands per primer varied from 19 to 27. Most of these bands (98.9%) were polymorphic among the species *Digitalis*. In general, polymorphic bands generated by RAPD-PCR using random primers had good merits for detecting DNA similarity and diversity between life organisms (Liu et al., 1998; McCormack et al., 2000; Nebauer et al., 2000; Geng et al., 2002).

The bandsharing values approach based on the presence or absence of amplified DNA bands was used to estimate similarity indices. In the present study, the average similarity index between catfish No. 5 and No. 9 showed the highest level within catfish population (0.945), whereas the similarity index between catfish No. 1 and No. 2 showed the lowest level (0.575). Also, the similarity index between bullhead No. 20 and No. 21 showed the highest level within catfish population (1.000), whereas the similarity index between bullhead No. 8 and No. 21 showed the lowest level (0.063). Overall, the similarity index between two populations was lower than that within population. In the concrete, the similarity index of catfish population was higher than that of bullhead population. All catfish individuals also revealed close genetic affinities, as compared with bullhead individuals, as summarized in Figure 1. It appears from the RAPD-PCR data that this large genetic difference in bullhead population may be genetic polymorphism.

Callejas and Ochando (1998) indicated that Spanish barbel species (*Barbus bocagei* and *B. graellsii)* were more related to each other than *B. sclateri* by means of the cluster analysis of the genetic similarity values obtained from RAPD data. The average level of bandsharing obtained by the five random primers used was 0.40±0.05 in the wild crucian carp population, contrast with 0.69±0.08 observed in the cultured crucian carp population (Yoon and Park, 2001). The degree of similarity in two carp species varied from 0.46 to 0.67 as calculated by bandsharing analysis (Yoon, 2001). The average level of bandsharing was approximately 0.57±0.03 between the species common carp and Israeli carp generated using various random primers.

The genetic relationships of 3 goat populations in China were studied by genetic differentiation coefficient and genetic similarity coefficient (Geng et al., 2002). The result showed that genetic relationship between LCG and CCG was the closest (genetic distance=0.0106), then CG and CCG (genetic distance=0.0109). They made mention that RAPD marker was more effective in analyzing the genetic relationship of populations. RAPD data analysis, including distance and parsimony methods, family clustering and the analysis of molecular variance, were applicable for the study of genetic relationships among species of the genus *Digitalis* (Nebauer et al., 2000). Namely, they stated that the species relationships revealed by RAPD-PCR approach were fully consistent with those previously obtained using morphological affinities. This result showed a similar tendency to that of Orozco-Castillo et al. (1994) that RAPD analysis reflected morphological differences between the sub-groups and morphological origin of the coffee material. The phenogram using RAPD data obtained across all primers revealed close relationships between accessions identities in native American maize accessions (Moeller and Schaal, 1999). The similarity index for Tamaroa white and Tamaroa/Tuscarora white was quite high (similarity=0.8). Also, they stated that Mandan red and Arikara bronze showed close genetic affinity, which confirmed historical records.

Generally speaking, the potential of RAPDs to identify diagnostic markers for strain, breed, species and population identification in fish (Johnson et al., 1994; Partis and Wells, 1996; Smith et al., 1997; Callejas and Ochando, 1998; Debenham et al., 2000; Hamm and Burton, 2000; Yoon, 2001), in livestock (Jeffreys and Morton, 1987; Welsh et al., 1991; Mohd-Azmi et al., 2000; Ramesha et al., 2002; Appannavar et al., 2003), in parasites (Dias Neto et al., 1993) and in plants (Orozco-Castillo et al., 1994; Vierling et al., 1994; Bommineni et al., 1997; Fischer et al., 2000) has also been demonstrated. In this study, RAPD-PCR analysis has revealed significant genetic distances between two species population pairs.

CONCLUSION

In conclusion, the similarity index of catfish population was higher than that of bullhead population. All catfish individuals also revealed close genetic affinities, as compared with bullhead individuals. High levels of genetic polymorphisms and the existence of population differentiation between two species showed RAPD-PCR approach is one of the most suitable tools for individuals and population biological DNA studies. Additionally, to obtain more reliable results, a larger number of sampling sites, increased sample sizes and other biological researches such as RFLP, AFLP, microsatellite and DNA sequencing may be required. Additional statistical analyses such as bootstrapping analysis and principal coordinates analysis based on the RAPD-PCR data need to be made to obtain further results.

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