발생과 생식 제 5권 제 2호, 151~158 (2001)
Dev. Reprod. Vol. 5, No. 2, 151~158 (2001)

Genetic Similarity in Crucian Carp(*Carassius carassius*) by PCR-RAPD Analysis

Jong-Man Yoon† and Jong-Yeon Kim[1]

Department of Marine Biomedical Science, College of Ocean Science and Technology, Kunsan National University, Kunsan, Jeollabuk-do 573-701, Korea

[1]Department of Marine Aquaculture and Biotechnology, College of Ocean Science and Technology, Kunsan National University, Kunsan, Jeollabuk-do 573-701, Korea

PCR-RAPD 분석에 의한 붕어(*Carassius carassius*)의 유전적 유사성

윤 종 만† · 김 종 연[1]

국립군산대학교 해양과학대학 해양생명의학과

[1]국립군산대학교 해양과학대학 해양생명양식학과

ABSTRACT : Genomic DNA from the blood of crucian carp(*Carassius carassius*) from lake and aquaculture facility in Kunsan, Korea was extracted in order to identify genetic differences by polymerase chain reaction-randomly amplified polymorphic DNAs(PCR-RAPD). Out of 12 primers, 6 generated 266 highly reproducible RAPD markers, producing approximately 2.1 polymorphic bands per primer in crucian carp from lake. The degree of similarity varied from 0.18 to 0.76 as calculated by bandsharing analysis in crucian carp from lake. The RAPD outlines obtained with DNA of two different crucian carp populations from Kunsan were different(0.47 from lake and 0.70 from aquaculture facility, respectively). The electrophoretic analysis of polymerase chain reaction-randomly amplified polymorphic DNAs(PCR-RAPD) products showed high levels of similarity between different individuals in crucian carp from aquaculture facility. This result implies the genetic similarity due to raising in the same environmental condition or inbreeding within the crucian carp from aquaculture facility in Kunsan. In other words, crucian carp may have high levels of genome DNA diversity due to the introduction of the wild population from the other sites of Kunsan even if it may be the geographical diverse distribution of this species. Generally, the RAPD polymorphism generated by these primers may be useful as a genetic marker for strain or population identification of important aquacultural fish species, crucian carp. However, in future, additional populations and sampling sites will be necessary to complement weak points.

Key words : Bandsharing analysis, Crucian carp, Genetic similarity, PCR-RAPD.

요 약 : 군산지역에 있는 호수와 양식장에서 채집된 붕어(*Carassius carassius*)의 혈액으로부터 추출된 genomic DNA를 무작위 primer를 이용한 PCR-RAPD 방법에 의해서 유전적 차이를 확인하고자 하였다. 12개 primer 중에서 6개를 이용한 결과 호수산 붕어의 경우 primer 당 약 2.1 polymorphic bands가 나타났고, 총 266개의 높은 RAPD marker가 확인되었으며, 0.18에서 0.76의 bandsharing 분석 결과가 나타났다. 군산지역에 있는 호수와 양식장에서 채취된 붕어 2집단간의 RAPD 특징을 bandsharing value로 비교 분석해 본 결과 각각 호수산이 0.47, 양식산이 0.70 으로 나타났으며, 이는 양식산 개체들간에 유사성이 높게 나타났다. 이러한 결과는 군산지역에 있는 양식장의 경우 유사한 환경조건내에서 붕어가 사육되었거나 혹은 오랜 기간동안 근친교배의 결과 이러한 유전적 유사성이 높게 나타난 것으로 사료된다. 달리 말하면 비록 다양한 지리적인 분포가 있더라도 군산지역의 다른 지역으로부터 야생산 붕어 집단의 도입으로 인하여 genomic DNA의 높은 수준의 다양성을 가질 수 있다는 것이다. 일반적으로 primer에 의해서 제시된 RAPD 다형성은 양식대상 어종이면서 온수성 어종인 붕어의 계통 혹은 집단을 확인하기 위한 유전적 표지인자로서 사용될 수 있을 것이다. 그러나 앞으로 집단 및 채집장소의 추가적인 확보 그리고 다른 방법을 통한 연구가 미비한 점을 보완할 수 있는 데 필요하다고 사료된다.

INTRODUCTION

Genetic markers by protein, enzyme and DNA electrophoresis had many potential applications in animal, plant and microbe

† Correspondence : Department of Marine Biomedical Science, College of Ocean Science and Technology, Kunsan National University. Miryong-dong Kunsan Jeollabuk-do 573-701 Korea. Tel: 82-63-469-1887, Fax: 82-63-463-9493, E-mail: jmyoon@kunsan.ac.kr

genetics and breeding research. The polymorphic DNA markers that were shown to genetically link to a trait of interest could be used for individual identification, gene cloning, pathological diagnostics, and for trait improvement in breeding programs.

There were so far used various molecular biological methods including restriction fragment length polymorphism(RFLP) (Beckenbach et al., 1990; Garcia-Mas et al., 2000) and randomly amplified polymorphic DNAs(RAPD) (Welsh & McClelland, 1990; Welsh et al., 1991; Deragon & Landry, 1992; Dias Neto et al., 1993; Nelson et al., 1993; Simpson et al., 1993; Orozco-Castillo et al., 1994; Vierling et al., 1994; Lilley et al., 1997; Garcia-Mas et al., 2000; McCormack et al., 2000) based on the polymerase chain reaction(PCR).

Geographical populations were mainly distinguished by RFLP -PCR method in various fishes(Wilson et al, 1987). Also, geographical populations were identified by RAPD-PCR method in various parasites(Bishop et al., 1993; Dias Neto et al., 1993). Especially, applications of RAPD to most fisheries had been at the levels of a few of fish species and crustacean apart from geographic sites(Smith et al., 1997; Tassanakajon et al., 1998; Hamm & Burton, 2000; McCormack et al., 2000). Random primers based on the polymerase chain reaction(PCR) also produced a higher percentage of polymorphic multiple-band outlines than other probes(Garcia-Mas et al., 2000). Genomic polymorphic bands generated by PCR amplification of DNA using arbitrary primers had some advantages for detecting DNA polymorphisms between organisms. Namely, the primer can detect polymorphisms in the presence or absence of specific nucleotide sequence information, and the polymorphisms can function as genetic markers(Smith et al., 1997).

In this study, DNA isolated from the blood was analyzed by 12 randomly amplified polymorphic DNA(RAPD) primers in order to identify genetic difference and develop the specific genetic markers in two crucian carp(*Carassius carassius*) populations from Kunsan.

MATERIALS AND METHODS

1. Blood collection

DNA samples of crucian carp(*Carassius carassius*) were obtained from a lake and an aquaculture facility in the periphery of Kunsan in Korea. There was approximately 50km the geographical distance between lake and aquaculture facility. RAPD analysis was performed on genetic DNA samples from a total of 24 crucian carp using 12 different random primers. The same DNA samples were used for each primer. The DNA samples collected from blood in two-year-old crucian carp populations. The fish were anaesthetized with MS 222(100 ppm). Blood samples were taken with sterile test tubes from the caudal vein into heparinized vials. The bloods obtained were refrigerated at 4℃ until use. All glassware, micropipette tips, microcentrifuge tubes and solutions were autoclaved to avoid DNA contamination.

2. Sources of genomic DNA

Samples of whole blood were placed into 10 ml heparinized vials, to which an 4 volumes of lysis buffer Ⅰ was added, and the mixture tube was gently inverted several times. The samples were incubated on ice for 30 min, centrifuged at 3,000 rpm for 10 min at 4℃ to pellet. The pellets were transferred to 1.5 ml Eppendorf tubes with lysis buffer Ⅰ, and then mixtures were centrifuged with microcentrifuge at 14,000 rpm for 1 min. The precipitates were dissolved with 0.8 ml lysis buffer Ⅱ. Samples were transferred to 1.5 ml Eppendorf tubes and added 15 μl proteinase K solution(10 mg/ml). The mixtures were gently inverted and incubated at 37℃ for overnight or 65℃ for 4 hrs. After incubation, 0.3 ml of 6 M NaCl were added and gently pipetted for a few min. 0.6 ml of chloroform were added to the mixture and then inverted(no phenol). Samples were spun down at 14,000 rpm for 5 min. The cleared lysates were extracted with ice-cold 70% ethanol, centrifuged at 14,000 rpm, and then precipitated. The DNA pellets were air-dried, and then dissolved in TE buffer. Purity was estimated by calculating the ratio of the absorbance(O.D. value) measured at 260~280 nm with a spectrophotometer(Beckman DU 600 series). The final concentration was estimated by agarose electrophoresis and EtBr staining.

3. Primer and marker

The primers(G+C content, 60~70%), designed for other purpose and chosen arbitrarily for these experiments, were obtained from Operon Technologies, USA(Table 1). Twelve decamer oligonucleotide primer sequences were used.

Table 1. Primers and primer sequences used for the detection of polymorphism in crucian carp(Carassius carassius)

Serial no of primer	Sequence(5' to 3')	GC content(%)	Serial no of primer	Sequence(5' to 3')	GC content(%)
OPA-01	CAGGCCCTTC	70	OPA-07	GAAACGGGTG	60
OPA-02	TGCCGAGCTG	70	OPA-08	GTGACGTAGG	60
OPA-03	AGTCAGCCAC	60	OPA-09	GGGTAACGCC	70
OPA-04	AATCGGGCTG	60	OPA-10	GTGATCGCAG	60
OPA-05	AGGGGTCTTG	60	OPA-11	CAATCGCCGT	60
OPA-06	GGTCCCTGAC	70	OPA-12	TCGGCGATAG	60

4. Amplification conditions

Amplification reactions were undertaken in volumes of 20 μl contained 10 ng of template DNA, 20 μl AccuPower premix (Bioneer Co., Korea) and 1.0 unit primer(Operon Technologies, USA). Amplification was performed in a DNA Thermal Cycler (Perkin Elmer, USA). This mixture was followed a predenaturation at 94℃ for 5 min. The thermal cycler programmed for 45 cycles at 94℃ for 1 min for denaturation, at 36℃ for 1 min for annealing, at 72℃ for 1 min for extension, at 72℃ for 5 min for post-extension. The amplified products were separated by electrophoresis in 1.4% agarose gels with TBE, detected by staining with ethidium bromide, illuminated with UV light and taken photographs by photoman system(Seoulin, Korea).

5. Analytical method

Bandsharing(BS) calculation was somewhat modified the formula of Jeffreys & Morton(1987) and Mohd-Azmi et al.(2000) : BS = 2(Bab)/(Ba+Bb). Where Bab is the number of bands shared by individuals a and b, Ba is the total number of bands for individual a, and Bb is the total number of bands for individual b. If compared between the three lanes, the formula would be: BS = 3(Nabc)/(Na+Nb+Nc) and so on. Only bands that were readily visible were scored. BS values in RAPD outlines were scored by the presence or absence of an amplification product at specific positions in the same gel.

RESULTS

1. Intra- and inter-population variations

Of the 12 arbitrarily selected primers, six random primers were used on the basis of the number and frequency of the polymorphisms producted. The bands in the molecular weight range from 0.07 to 1.35 kilobase pairs generated by random primer OPA-2(TGCCGAGCTG) were observed in crucian carp population from lake(Fig. 1). There were showed genomic DNA polymorphic bands generated using a primer(70% of GC contents) to amplify DNA isolated from the blood of 11 individuals. The specific band pattern showing DNA polymorphism was observed in 0.12 kb. The RAPD profiles in crucian carp population from lake obtained by a primer(OPA-3, AGTCAGCCAC) with DNA of individuals were some different(Fig. 2). The identical band pattern of PCR-RAPD products was observed in 0.28 and greater than 0.31 kb, respectively. The intra-population

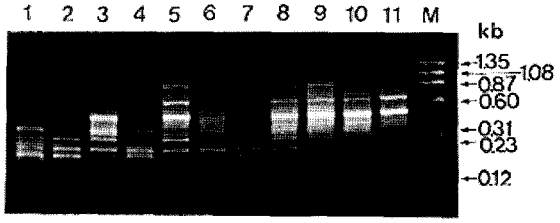

Fig. 1. Individual specific RAPD patterns of crucian carp from lake amplified by arbitrary OPA-2(TGCCGAGCTG). Amplification products were electrophoresed on a 1.4% agarose gel with TBE(0.09 M Tris, pH 8.5, 0.09 M boric acid, 2.5 mM EDTA) and detected by staining with ethidium bromide. Each lane(1~11) shows different individual DNA samples. M: Molecular size standard(ϕX174 DNA marker digested with *Hae*III).

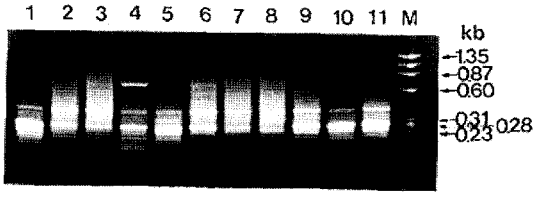

Fig. 2. Specific RAPD patterns of crucian carp from lake amplified by arbitrary primer OPA-3(AGTCAGCCAC). Each lane(1~11) shows different individual DNA samples. M: ϕX174 DNA marker digested with *Hae*III.

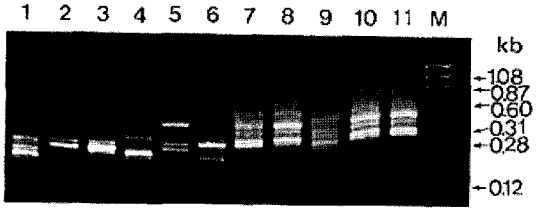

Fig. 3. Individual specific RAPD patterns in crucian carp from lake amplified by arbitrary OPA-5(AGGGGTCTTG). Each lane(1~11) shows different individual DNA samples. M: Molecular size marker(Φ ×174 DNA marker digested with *Hae*Ⅲ).

variation was revealed in the band patterns ranged from greater than 0.6 to 0.87 kb. Another primer OPA-5(AGGGGTCTTG) also generated RAPD band patterns showing DNA polymorphisms in crucian carp population from lake(Fig. 3). The similar band pattern was observed from 0.28 to 0.31 kb. The Intra-population variation was revealed in the band patterns ranged from 0.31 to 1.08 kb, producing a specific band RAPD profile in 0.12 kb. The differences between populations should be diagnostic of specific strains and their relatedness. Also, the bands from 0.19 to 2.03 kilobase pairs generated by random primer OPA-7 (GAAACGGGTG) were observed in crucian carp population from lake(Fig. 4). This primer yielded three single-band profiles (lanes 1, 2 and 7). The Intra-population variation was revealed in the band patterns ranged from 0.28 to 2.03 kb. Another primer, OPA-10(GTGATCGCAG), detected a few of RAPD major bands, approximately from greater than 0.6 to 1.35 kb in crucian carp population from lake(Fig. 5). The DNA bands of from greater than 0.31 to less than 0.60 kb were present in every individuals. A band of 1.35 kb and the other of 1.08 kb were

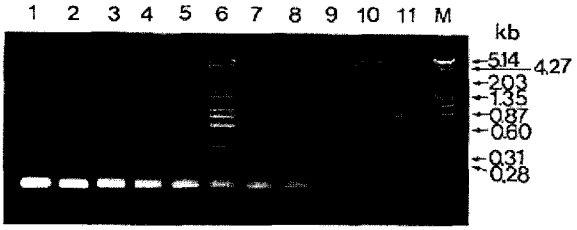

Fig. 4. PCR-RAPD products of crucian carp from lake amplified by arbitrary OPA-7(GAAACGGGTG). Each lane(1~11) shows different individual DNA samples. The primer produced three single-band RAPD profiles(lanes 1, 2 and 7). M: Molecular size standard(Φ ×174 DNA marker digested with *Hae*Ⅲ and Lambda DNA marker/ *Eco*R Ⅰ + *Hind*Ⅲ).

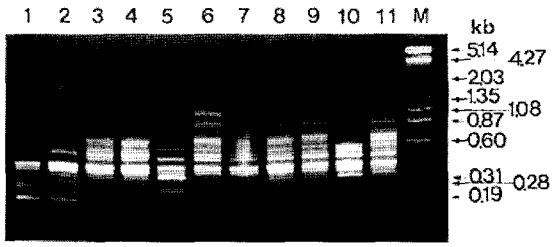

Fig. 5. Individual specific RAPD patterns in crucian carp from lake amplified by arbitrary OPA-10(GTGATCGCAG). Each lane(1~11) shows different individual DNA samples. M: Molecular size marker(Φ ×174 DNA marker digested with *Hae*Ⅲ and Lambda DNA marker/ *Eco*R Ⅰ + *Hind*Ⅲ).

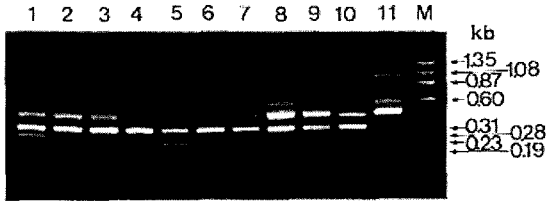

Fig. 6. RAPD profiles in crucian carp from lake amplified by primer OPA-12(TCGGCGATAG). Each lane(1~11) shows eleven individuals used. M: Molecular size marker(Φ ×174 DNA marker digested with *Hae*Ⅲ).

present in only 1 of 11 individuals, respectively, which were polymorphic. The RAPD polymorphism generated by random primer OPA-12(TCGGCGATAG) showed polymorphic bands in crucian carp population from lake(Fig. 6). The sizes of the bands recorded ranged from 0.23 kb to approximately 1.35 kb. There were showed genomic DNA polymorphic bands generated using a primer(OPA-12, 70% of GC contents) to amplify DNA isolated

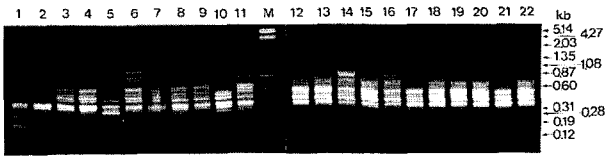

Fig. 7. Specific RAPD polymorphic bands generated in two crucian carp populations by arbitrary primer OPA-10(GTGATCGCAG). Each lane(1~22) shows different individual DNA samples(lanes 1~11 from one lake in kunsan and lanes 12~22 from the other aquaculture facility in Kunsan). M: Two standard markers(Φ ×174 DNA marker digested with Hae Ⅲ and Lambda DNA marker/*Eco*R Ⅰ+*Hind* Ⅲ).

from the blood of 11 individuals in crucian carp population from lake.

The RAPD polymorphism generated by random primer OPA -10 showed polymorphic bands in two crucian carp populations (Fig. 7). In this range from approximately 0.19 to 1.35 kb the patterns were highly reproducible and also very different between two populations. However, this primer produced the sizes of polymorphic DNA bands ranged from 0.6 to 1.35 kb and from 0.19 to 0.31 kb, respectively. The band exhibited the inter -population-specific characteristics.

2. Bandsharing scores

In six primers of the 12 RAPD primers used, the number of bands produced per primer varied from 1 to 15 with an average of 8.3 in crucian carp from lake(Table 2). Six primers produced amplified fragments which were consistently polymorphic. A total of 549 amplified products were produced of which 266 were polymorphic(48.5%). 3.8 of the 44.3 amplified bands were found to be polymorphic in crucian carp from lake(Table 3). Also, about 8.6% of total polymorphic bands were either specific to crucian carp from lake as summarized in Table 3. Especially, primer OPA-2 generated the highest number of fragments among the primers used with the average of 6.0 in crucian carp from lake. The degree of similarity varied from 0.18 to 0.76 as calculated by bandsharing analysis. Also, the average level of bandsharing was 0.51±0.08 within the crucian carp populations from lake. In addition, the RAPD outlines obtained with DNA of different crucian carp populations between from lake and from aquaculture facility in Kunsan were different(Table 4). Especially, the RAPD outlines obtained with DNA of crucian carp population of a site(lanes 1~11 from lake) were much more varied than those of the other(lanes 12~22 from aquaculture facility). A number of polymorphic bands were identified in two crucian carp populations(Fig. 7, Table 4).

Table 2. Number of RAPD bands for single primer using agarose gel in crucian carp(*C. carassius*) from lake in Kunsan

Primers no	Number of bands	Total bands	Average
OPA-2	9~14	133	12.1
OPA-3	5~10	67	6.1
OPA-5	6~11	81	7.4
OPA-7	1~14	61	5.5
OPA-10	11~15	126	11.5
OPA-12	3~10	81	7.4
Total		549	50
Average	5.8~12.3	91.5	8.3

Table 3. Bandsharing(BS) within the crucian carp population from lake(mean or mean±S.E)

Primers no	Total of polymorphic bands	Average number of polymorphic bands	BS values
OPA-2	66	6.0	0.50
OPA-3	25	2.3	0.76
OPA-5	23	2.1	0.68
OPA-7	61	4.1	0.18
OPA-10	57	5.2	0.52
OPA-12	34	3.1	0.41
Total	266	22.8	3.05
Average	44.3	3.8	0.51±0.08

Table 4. Comparisons of the RAPD profiles of two crucian carp populations in Kunsan(lanes 1~11 from lake, lanes 12~22 from aquaculture facility) obtained with arbitrary primer OPA-10 (GTGATCGCAG)

Lane no	Number of bands	Total bands	Polymorphic bands	BS value
1~11*	9~15	117	48	0.47
12~22**	8~13	94	20	0.70
Average	8.5~14	105.5	34	0.59

* : from lake

** : from aquaculture facility

DISCUSSION

The amplified products can be generated as DNA polymorphic bands by gel electrophoresis, the patterns being characteristic of both the primers and the template DNAs. The PCR-RAPD method can be applied to identify genetic similarity and diversity in crucian carp(*C. carassius*) using twelve different primers. Many authors could detect genetic polymorphisms in a wide variety of organisms by PCR-RAPD using a large number of different primers(Welsh & McClelland, 1990; Welsh et al., 1991).

In this study, it was used DNA extracted from blood of crucian carp populations which have the genome size of fragments

from approximately 120 to 2,030 bp as shown in Figs. 1~7. The number and size of the fragments generated strictly depend on the nucleotide sequence of the primer used and on the source of the template DNA, resulting in a genome-specific polymorphic band of random DNA fragment. Six primers generated 266 highly reproducible RAPD markers, producing approximately 2.1 polymorphic bands per primer in crucian carp from lake as summarized in Table 3. Especially, on average, 34 of the 105.5 amplified bands were found to be polymorphic between two crucian carp populations as summarized in Table 4.

For these results, there were population-specific RAPD fragments in crucian carp and there were differences in frequencies of six primer fragments, as have been reported in catfish(Liu et al., 1998), livestock(Koh et al., 1997) and sorghum (Vierling et al., 1994). Some intra-specific or intra-strain RAPD variations were also observed for different strains or for individuals(Welsh et al., 1991; Liu et al., 1998). Particularly, Liu et al.(1998) screened a collection of RAPD markers in catfish and identified 22 primers that revealed 171 strain-specific genetic markers. Also, Johnson et al.(1994) referred a collection of RAPD markers in zebrafish and identified 116 primers that revealed 721 strain-specific genetic markers. McCormack et al.(2000) identified that a minimum of 93% of genotypic variance occurred among individuals within populations. These workers have found more variation within a strain or population than between strains or populations considered as a whole. Especially, in marine organism, 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.

Bandsharing scores were calculated as an expression of similarity of RAPD polymorphic bands of animals from either the same or different breeds(Jeffreys & Morton, 1987; Mohd -Azmi et al., 2000). In this study, primer OPA-2 generated the highest number of fragments among the primers in crucian carp from lake(the total of 133, the average of 12.1). This specific primer was also found to be useful in the individual identification of crucian carp, resulting from the different DNA polymorphism among individuals the same as result of Liu et al.(1998) obtained in catfish species. RAPD markers produced by primers were effective in determining polymorphism between sorghum lines(Vierling et al., 1994). In this study, it was identified that about 8.6% of total amplified bands were specific to crucian carp. This result demonstrates the utility of the technique in strain or breed identification(Welsh et al., 1991; Bishop et al., 1993; Simpson et al., 1993; Dias Neto et al., 1993; Vierling et al., 1994; Lilley et al., 1997; Liu et al., 1998). The potential of RAPDs to identify diagnostic markers for strain or species identification in mice(Welsh et al., 1991), in parasites or pathogens(Welsh & McClelland, 1990; Bishop et al., 1993; Simpson et al., 1993; Dias Neto et al., 1993; Lilley et al., 1997), in livestock(Koh et al., 1998), in plants(Deragon & Landry, 1992; Nelson et al., 1993; Orozco-Castillo et al., 1994) and in fish(Johnson et al., 1994; Liu et al., 1998; Yoon, 1999) has also been demonstrated. In addition to mapping and breeding applications, PCR-RAPD system could be very useful for the rapid certification and quality control of seed production and for all projects based on PCR amplification of specific plant DNA fragments(Deragon & Landry, 1992).

In this study, the RAPD outlines obtained with DNA of two different crucian carp populations from Kunsan were different (0.47 in population from lake and 0.70 in population from aquaculture facility). There was significant difference between two populations apart from each other. However, this result that a number of polymorphic bands in a site were identified, implies the genetic variation or diversity due to introduction within the crucian carp populations from different geographical regions compared with the other. On the one hand, this genetic diversity in a crucian carp population may have been caused by a transitory increase in broodstock numbers in the hatcheries from which Kunsan population was derived. In other words, crucian carp may have high levels of genome DNA diversity due to the introduction of the wild population from the other sites of Kunsan even if it may be the geographical diverse distribution of this species. On the other hand, this result may imply the genetic similarity due to raising in the same environmental conditions or to inbreeding within the crucian carp from Kunsan. However, genetic distance was found to be independent of geographic distance over the long-distance(300 km) sampling range(Hamm & Burton, 2000).

In the present study, this author demonstrated the successful use of RAPD analysis to identify population differentiation of crucian carp. However, further analysis is required to identify

primers that amplify sufficient bands shared by the species to permit a quantitative analysis. Additionally, it seemed to be essential to get better data on the genetic distances between a number of crucian carp populations.

ACKNOWLEDGEMENTS

The authors are grateful to the Fisheries Science Institute of Kunsan National University for equipment and funding in the program year of 2002.

REFERENCES

Beckenbach AT, Thomas WK, Sohrabi H (1990) Intraspecific sequence variation in the mitochondrial genome of rainbow trout (*Oncorhynchus mykiss*). Genome 33:13-15.

Bishop RB, Sohanpal B, Morzaria S (1993) Theileria parva: Detection of genomic polymorphisms by polymerase chain reaction amplification of NA using arbitrary primers. Exp Parasitol 77:53-61.

Deragon JM, Landry BS (1992) RAPD and other PCR-based analyses of plant genomes using DNA extracted from small leaf disks. PCR Methods and Applications 1:175-180.

Dias Neto E, Steindel M, Passos LKF, Pereira de Souza C, Rollinson D, Katz N, Romanha AJ, Pena SDJ, Simpson AJG (1993) The use of RAPDs for the study of the genetic diversity of *Schistosoma mansoni* and *Trypanosoma cruzi*. DNA Fingerprinting:339-345.

Garcia-Mas J, Oliver M, Gomez-Paniagua H, Vicente MC (2000) Comparing AFLP, RAPD and RFLP markers for measuring genetic diversity in melon. Theor Appl Genet 101:860-864.

Hamm DE, Burton RS (2000) Population genetics of black abalone, *Haliotis cracherodii*, along the central California coast. J Exp Mar Biol Ecol 254:235-247.

Jeffreys AJ, Morton DB (1987) DNA fingerprints of dogs and cats. Anim Genet 18:1-15.

Johnson SL, Midson C, Ballinger EW, Postlethwait JH (1994) Identification of RAPD primers that reveal extensive polymorphisms between laboratory strains of zebrafish. Genomics 19:152-156.

Koh MC, Lim CH, Chua SB, Chew ST, Phang ST (1997) Random amplified polymorphic DNA (RAPD) fingerprints for identification of red meat animal species. Meat Sci 48: 275-285.

Lilley JH, Cerenius L, Söderhäll K (1997) RAPD evidence for the origin of crayfish plague outbreaks in Britain. Aquaculture 157:181-185.

Liu Z, Li P, Argue BJ, Dunham RA (1998) Inheritance of RAPD markers in channel catfish (*Ictalurus punctatus*), blue catfish (*I. Furcatus*) and their F_1, F_2 and backcross hybrids. Anim Genet 29:58-62.

McCormack GC, Powell R, Keegan B (2000) Comparative analysis of two populations of the brittle star *Amphiura filiformis* (Echinodermata: Ophiuroidae) with different life history strategies using RAPD markers. Mar Biotechnol 2:100-106.

Mohd-Azmi M, Ali AS, Kheng WK (2000) DNA fingerprinting of red jungle fowl, village chicken and broilers. Asian-Aus J Anim Sci 13:1040-1043.

Nelson CD, Nance WL, Doudrick RL (1993) A partial genetic linkage map of slash pine (*Pinus elliottii* Engelm. var. *elliottii*) based on random amplified polymorphic DNAs. Theor Appl Genet 87:145-151.

Orozco-Castillo C, Chalmers KJ, Waugh R, Powell W (1994) Detection of genetic diversity and selective gene introgression in coffee using RAPD markers. Theor Appl Genet 87:934-940.

Simpson AJG, Dias Neto E, Steindel M, Caballero OLSD, Passons LKJ, Pena SDJ (1993) The use of RAPDs for the analysis of parasites. DNA Fingerprinting:331-337.

Smith PJ, Benson PG, McVeagh SM (1997) A comparison of three genetic methods used for stock discriminaion of orange roughy, *Hoplostethus atlanticus*: allozymes, mitochondrial DNA, and random amplified polymorphic DNA. Fish Bull 95:800-811.

Tassanakajon A, Pongsomboon S, Jarayabhand P, Klinbunga S, Boonsaeng V (1998) Genetic structure in wild populations of black tiger shrimp (*Penaeus monodon*) using randomly amplified polymorphic DNA analysis. J Mar Biotechnol 6:249-254.

Vierling RA, Xiang Z, Joshi CP, Gilbert ML, Nguyen HT (1994) Genetic diversity among elite *Sorghum* lines revealed by restriction fragment length polymorphisms and random

amplified polymorphic DNAs. Theor Appl Genet 87:816-820.

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18:7213-7218.

Welsh J, Petersen C, McClelland M (1991) Polymorphisms zgenerated by arbitrarily primed PCR in the mouse: application to strain identification and genetic mapping. Nucleic Acids Res 19:303-306.

Wilson GM, Thomas WK, Beckenbach AT (1987) Mitochondrial DNA analysis of Pacific northwest populations of *Oncorhynchus tshawytscha*. Can J Fish Aquat Sci 44:1301-1305.

Yoon JM (1999) Genomic polymorphisms of genome DNA by polymerase chain reaction-RAPD analysis using arbitrary primers in rainbow trout. Korean J Anim Reprod 23:303-311.