Iranian Journal of Fisheries Sciences 8**(1)** 47-56 2009

Inheritance of RAPD marker in female grass carp (*Ctenopharyngodon idella*), male bighead carp (*Hypophthalmichthys nobilis*) and their F1 hybrids

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Received: October 2005 Accepted: May 2008

Abstract

Genetic variation and inheritance of randomly amplified polymorphic DNA (RAPD) markers in female grass carp *(Ctenopharyngodon idella)* and male bighead carp *(Hypophthalmichthys nobilis)* and their F1 offspring hybrid have been studied. For this purpose, genomic DNA was extracted from muscle tissue according to phenol-chloroform method, and six decamer primers were used for amplifying polymorphic DNA. Results from 77 produced bands showed that all RAPD bands pattern of parents were present in F1 offspring hybrid, which indicated the high influence and dominance of the mentioned markers. The low difference in polymorphisms in used markers between F1 offspring hybrid and parents shows that parents are heterozygous in some loci, which can cause low difference in hybrids of two distinct genera. Although our results revealed that RAPD markers had a suitable efficiency in distinguishing parents from F1 hybrids, genetic diversity of triploid and diploid F1 hybrid were not detected by introduced primers.

Keywords: RAPD marker, Grass carp, Bighead carp, Hybridization, Triploidy

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Introduction

Cyprinidae is commercially regarded as the most important fish family, contributing the greatest share to world's farmed fish production and some of them such as grass carp considered as an aquatic weed biocontroller (Sutton, 1981; Sutton & Vandiver, 1997). Grass carp, *Ctenopharyngodon idella,* is one of the Chinese carps which was introduced to the Iranian freshwater bodies in 1966. It has been allotted about 10-15 percent of warm water polyculture. Female grass carp, and male bighead carp, *Hypophthalmichthys nobilis*, were first crossed in Russia (Aliev, 1967) and later in Hungary (Marian & Krasznai, 1982) and USA (Beck *et al*., 1980) to produce a viable intergeneric hybrid. The Hungarian researchers reported that all their hybrids were triploid. Such a triploid hybrid would, in theory, be sterile because the extra set of chromosomes from female grass carp would lead to the failure of gonad development or the production of aneuploid gametes. This hybrid could be, therefore, released into lakes and canals for aquatic weed control without risk of reproduction. The ploidy of grass carp and bighead carp hybrids should be determined before they are released into water bodies or used for research. This can be achieved by chromosomal counts or measurements of erythrocyte nuclear volume (Beck & Biggers, 1983a; Linhart *et al*., 2001) and nucleic-acid fluorescence of erythrocytes (Allen & Stanley, 1983). These are accurate but time consuming methods and often require sacrifice of the fish. RAPD procedure, however, does not demand previous knowledge of the genome and requires minor amounts of template DNA; besides, complete genome can be screened and genetic polymorphism can be visualized within 24h from genomic extraction and, finally, the storage of material is easy (Williams *et al*., 1990).

Recently grass carp and bighead carp have been used for hybridization and sterilization purposes in Iran (Kalbassi *et al.*, 2004). The primary interest in this hybrid was its potential for biological control of noxious aquatic vegetation such as Azolla. Due to morphological similarities of F1 hybrids at larval stage, the main objective of the present study was, therefore, to establish the molecular identification and verification of the hybrids by using randomly amplified polymorphic DNA (RAPD) for the first time.

Materials and methods

Experimental fish were provided from previous study (Kalbassi *et al.*, 2004) and raised at Fish Breeding and Culture Center of Shahid Rajaee in Sari, Mazandaran Province. A total of 24 individuals from each species (parents and hybrids) were applied for the experiments. Specimens were dissected *in situ;* muscle was frozen, transferred to the laboratory in liquid nitrogen and stored at –80°C until DNA extraction.

Genomic DNA was extracted from 50-100mg of muscle tissue as described by Hillis

et al. (1996). Samples were incubated with sodium dodecyle sulphate (SDS) and genomic DNA was purified using phenol: chloroform extraction and ethanol precipitation. The resulting pellets were washed in 70% ethanol, dried and resuspended in10mM Tris (pH 8.0), 1mM EDTA.

After adjustment of DNA concentration (Fig. 1), to generate RAPD profiles from parents and their hybrids, totally six oligodecamers from MWG Oligosynthesis Ltd. (USA) were used (Table 1). DNA amplification reactions were performed under conditions reported by Williams *et al*. (1990).

PCR products were separated by 1.5% agarose gel electrophoresis containing ethidium bromide and TAE (40mM Tris, 20mM acetic acid, 1mM EDTA) buffer system (Maniatis *et al*., 1995).

Gels were photographed under UV light using a Polaroid camera. Band sizes were estimated using a 100 base pairs ladder (Roche) standard.

All amplification reactions were carried out, at least, twice in order to score reproduce-ble bands clearly. 98% of amplified bands were routinely repeatable and non-reproducible bands, generally weaker, were excluded from the analysis. All PCR products were detected on 1.5% agarose gel by silver staining of 6% polyacrylamid gel electro-phoresis (Maniatis *et al*., 1995).

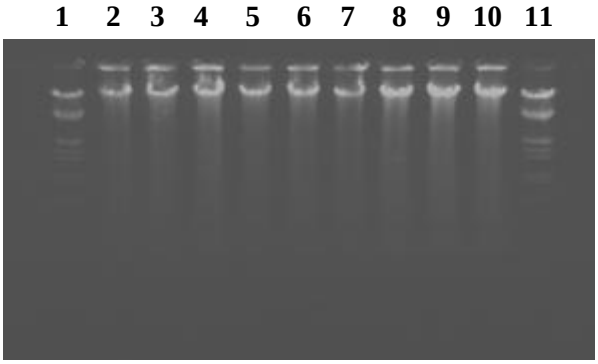

Figure 1: Genomic DNA of hybrids and grass carp and bighead carp; Lanes 1 and 11: marker, Roche; Lane 2: grass carp, lane 3: bighead carp and lane 4-10 F1 hybrids.

Table1: Sequences of used decamer primers from MWG Oligosynthesis Ltd. (USA)

Primers	Sequences
1	5´-GGGACGATGG-3´
2	5´-AGTGCGCTGA-3´
3	5´-GAGCGCCTTG-3´
4	5´-TCTCCGCAAC-3´
5	5´-AGTAGGGCAC-3´

6	5´-ACATCGCCCA-3´

Results

The primers used in the present study, amplified a total of 37 and 40 bands in grass carp and bighead carp, respectively, which ranged in size from 150-1500bp. In grass carp, 36.6% of these bands were polymorphic (present in at least 95% of all investigated individuals) and 63.4% were monomorphic (present in less than 95%). In bighead carp, 30.07% were polymorphic and 69.93% were monomorphic. Each primer generated 3-10 scorable bands. The complexity of the banding patterns varied dramatically between primers. Primer 6 gave the highest number of amplified bands (10 bands) but yielded no polymorphic level (less than 50%) (Fig. 2).

The six primers amplified an average of 6/1 bands from grass carp and 6/6 bands from bighead carp. About 30-36% of the amplified bands from grass carp and bighead carp were either specific to grass carp or bighead carp. A total of 1850 bands were amplified from six RAPD primers, of which 264 bands were specific to grass carp and 269 were specific to bighead carp (for all samples).

Almost all RAPD bands from both parents were found in RAPD profiles of F1 hybrids (Fig. 3), indicating high penetrate and dominant nature of RAPD markers. Very low levels of segregation of polymorphic RAPD markers were observed in F1 individuals, indicating the heterozygous nature of some individuals at the RAPD marker loci in the parents, consistent with low levels of intraspecific polymorphism.

Ag-staining of 6% polyacrylamid gel electrophoresis of producing profile of primer 4 in hybrids grass carp and bighead carp showed no differences between diploid hybrid and triploid hybrid (Fig. 4); minimum differences, which were due referring to individual variation, were not reproducible.

DNA templates from 72 sampled fish (24 from each group of grass carp, bighead carp and their hybrids) isolated at different times were tested (2-4 replicate) for reproducibility. Exact reproducibility was observed when similar concentrations of DNA template were utilized. Variation in number of amplified bands was observed with drastic changes (greater than 1000-fold) in DNA template concentration. Similarly, different numbers of bands was amplified with drastic changes (greater than 10-fold) in primer concentrations. Generally, higher DNA template concentration and primer concentration led to amplification of more bands, making scoring difficult. However, when consistent quantities of DNA and primers were used (25-50ng DNA sample and 20 picomol of primer), consistent and reproducible results were obtained.

Another factor for reproducibility of RAPD bands was the size of amplified products. Generally, amplified products with large sizes (more than 2kb) showed low reproducibility.

Good reproducibility was obtained with bands ranged from 150-1500bp.

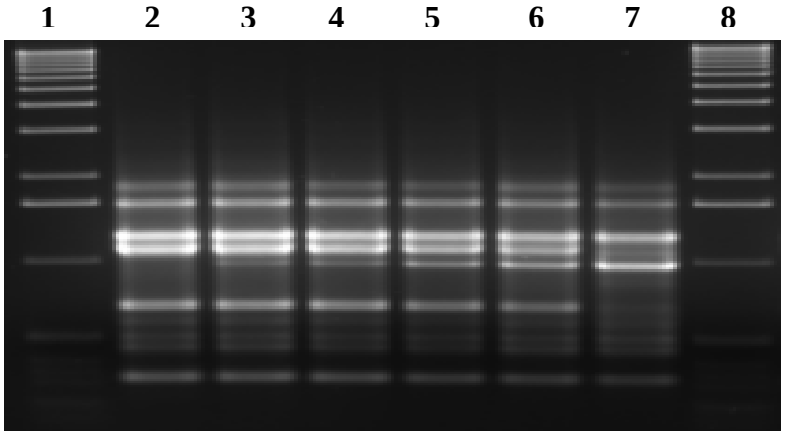

Figure 2: Producing profile of primer 6 in hybrids, grass carp and bighead carp. This figure shows no difference among species. Lines 1 & 8: 100bp ladder; 2, 6, 7: triploid hybrid; 3: grass carp, 4: diploid hybrid; 5: bighead carp.

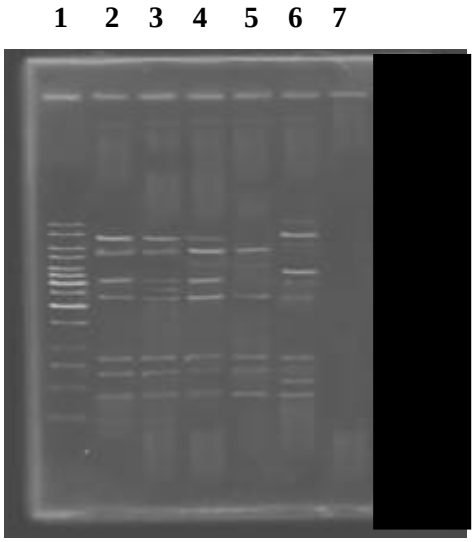

Figure 3: PCR-RAPD profile produced with primer 1 show the differences among grass carp, bighead carp and their F1 hybrid. From left to right: 1: 100bp ladder, 2: diploid hybrid, 3,4: triploid hybrid, 5: grass carp, 6: bighead carp, 7: negative control.

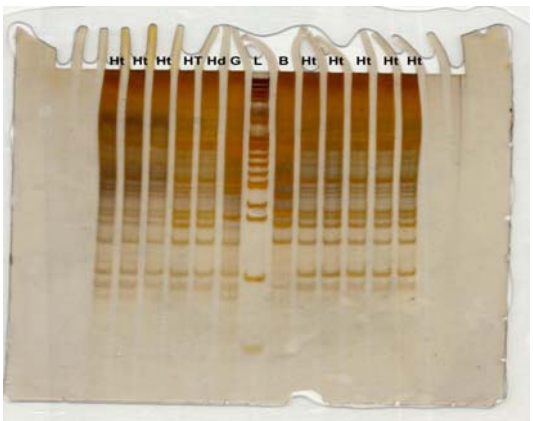

Figure 4: Ag-staining of 6% polyacrylamid gel electrophoresis of produced profile of primer 4 in hybrids, grass carp and bighead carp. (HT=triploid hybrid; HD:diploid hybrid; G:grass carp; B: bighead carp).

Discussion

RAPD generated a large number of polymorphic DNA bands between grass carp and bighead carp without requiring any previous knowledge of cyprinid genome, thus making it an efficient system for generating DNA markers using the interspecific hybrid system. Only one primer of six primers could not detect difference between grass carp and bighead carp (primer no. 6). RAPD profiles among species or strains provide an efficient system to generate molecular marker for gene mapping in an intraspecific mating plan. This partially explains why RAPD is more popular in research of plants and microorganisms, where interspecific hybrids are often available for use in mapping analysis.

According to Demeke *et al.* (1992) and Huff *et al.* (1993), six to seven primers were sufficient to assess genetic variation within and among population of highly polymorphic species. We, used six primers to identify molecular variation of grass carp and bighead carp and their F1 hybrids, and our results revealed that it was sufficient to detect differences between them.

Most profiles of our primers revealed that the major observed bands in parents were also found in hybrids. It may be due to high power of RAPD markers in many bands which had mendelian or dominant inheritance, which could be found in homozygote alleles forms in their parents. Also, as the entire hybrid parents belonged to the same population, they had many similar alleles which showed high similarity in hybrid profile patterns in these primers.

The parental species (*C. idella* and *H. nobilis*) showed morphologically distinct differences in our previous study (Kalbassi *et al.*, 2004). In the other hand interspecific hybrids usually exhibit a complex mixture of morphological characteristics inherited from dam and sire. Therefore, final results within F1, presented a wide polymorphism involving morphological and meristic traits. As shown by Beck and Biggers (1982), offspring produced by hybridizing *C. idella* with *H. nobilis* can yield diploid and triploid individuals. The karyological analysis of two hybrids revealed a triploid number of 3n=72 chromosomes (Beck & Biggers, 1982). Ploidy was also verified by the measurement of the largest erythrocyte axis. It is also known from the literature that hybridization between *C. idella* and *H. nobilis* produces offsprings in which a significant percentage is of triploid hybrids (Allen & Wattendrof, 1987). Beck and Biggers (1983b) reported that diploid *C. idella* and *H. nobilis* hybrids are more likely to be deformed and stunted in growth than triploids. This fact might cause a higher mortality of diploid individuals during their development,

which could add to a bias in the ratio between triploid and diploid individuals in the offspring.

The genome composition analysis of the hybrids could be a better tool for the detection of the genetic characteristics of hybrids and their parents. Although Williams *et al.* (1990) reported that RAPD marker was the dominant marker, there are reports for co-dominant characters of RAPD marker. Liu *et al.* (1998) applied RAPD marker for detection of genetic difference of channel catfish (*Ictalurus punctatus*), blue catfish (*I. furcatus*) and their F1, F2 and backcross hybrids. The mentioned RAPD marker was found to be a powerful technique for showing high genetic difference of channel catfish, blue catfish and their F1, F2 hybrids. In this study, the bands with molecular size higher than 2000bp showed no reproducibility and, therefore, were deleted from result and bands with 150-1500bp were considered in results. Liu *et al.* (1999) also applied this range of band size to obtain their results. Lack of observation of differences between diploid and triploid hybrids could be attributed to the inability of the RAPD marker to show recessive alleles. Minimum differences between diploid and triploid samples could, probably, be attributed to little individual differences. Also we could not see any differences even after pooling of hybrids DNA (Fig. 4). Soraia *et al.* (2005) applied SPAR (single primer amplification reaction), but they failed to detect any difference between triploid and diploid hybrids of grass carp and bighead carp through this method. They, therefore, suggested that FISH (Fluorescence in situ hybridization), quantities PCR and flowcytometery to be tested for the future studies.

In conclusion, final results of this study revealed that RAPD technique could be applied as a suitable and easy method for detecting molecular differences between parents (grass carp and bighead carp) and their hybrids, specially in small fish which their identification are hard or time consuming, without a need to sacrifice the fish, but genetic diversity of triploid and diploid F1 hybrid was not detected by our primers.

References

Allen, S.K. and Stanley, J.G., 1983. Ploidy of hybrid grass carp bighead carp determined by flow cytometry. Transactions of American Fisheries Society. **112:**431-435.

Allen, S.K. and Wattendorf, R.J., 1987. Triploid grass carp: Status and management implications. Fisheries. 12**(4):**20-24.

Beck, M.L., Biggers, C.J. and Dupree, H.K., 1980. Kariological analysis of *Ctenopharyngodon idella, Aristichthys nobilis,* and their F1 hybrid. Transactions of the American Fisheries Society. **109:**433-438.

Beck, M.L. and Biggers, C.J., 1982. Chromosomal investigation of *Ctenopharyngodon idella Aristichhys nobilis*. Experientia. 38**(3):**319.

Beck, M.L. and Biggers, C.J., 1983a. Erythrocyte measurements of diploid and triploid C*tenopharyngodon idella* and *Hypophthalmichthys nobilis* hybrids. Journal of Fish Biology. **22:**497-502.

Beck, M.L. and Biggers, C.J., 1983b. Ploidy of hybrids between grass carp and bighead carp determined by morphological analysis. Transactions of American Fisheries Society. **112:**808-811.

Demeke, T., Adams, R.P. and Chibbar, R., 1992. Potential taxonomic use of random polymorphic DNA (RAPD): A case study in Brassica. Theory of Applied Genetics. **84:**990-994.

Hillis, D., Moritz, C. and Barbara, M.K., 1996. Molecular Systematic, 2nd ed., Sinauer Associates Inc. 276P.

Kalbassi, M.R., Ebrahimzadeh, S.M., Nazari R.M. and Behrozi, Sh., 2004. Comparison of some biological parameters between grass carp and female grass carp × male bighead carp hybrid. The 7th Asian Fisheries Forum, November 2004, Penang, Malaysia.

Linhart, O., Haffray, P., Ozouf-costaz, C., Flajshan, S.M. and Vandeputte, M., 2001. Comparison of methods for hatchery-scale triploidization of European catfish (*Silurus glanis* L.). Journal of Applied Ichthyology. 17**(6):**247-286.

Liu, Z.J., Li, P., Argue, B.J. and Dunham, R.A., 1998. Inheritance of RAPD markers in channel catfish (*Ictalurus punctatus*), blue catfish (*I. furcatus*), and their F1, F2 and backcross hybrids. Animal Genetics. **29:**58-62.

Liu, Z.J., Li, P., Argue, B.J. and Dunham, R.A., 1999. Random amplified poly-morphic DNA markers: Usefulness for gene mapping analysis of genetic variation of catfish. Aquaculture. **174:**59-68.

Maniatis, T., Frisch, P. and Sambrook, J., 1995. Molecular cloning. A laboratory manual. Cold Spring Harbor. New York, USA. 320P.

Marian, T. and Krasznai, Z., 1982. Cross breeding of grass carp (*Ctenopharyngodon idella*) and bighead (*Aristichthys nobilis*) embryogenesis of interspecific hybrid and results of its morphological analysis. Aquacultura Hungarica (Szarvas). **3:**5-15.

Soraia, B.A., Fernandes, F.M., Lopes, C.E., Kulikoski, R., Kulikoski, J.M. and Almeida-Toledo, L.F. , 2005. Molecular marker in Chinese carps and their interspesific hybrids. Genetics and Molecular Biology. 28**(1):**172-174.

Sutton, D.L., 1981. Grass carp hybridization and observation of a grass carp and bighead carp hybrid. Journal of Aquatic Plant Management. **199:**37-39.

Sutton, D.L. and Vandiver, V. JR., 1997. Grass carp, a fish for biological management of hydrilla and other aquatic weeds in Florida, University of Florida, Agricultural Experiment Station, Institute of Food and Agricultural Sciences Bulletin. **867:**12.

Williams, J.G.K., Kubelik, A.R., Liva, K., Rafalski, J.A. and Tingej, S.V., 1990. DNA polymorphism amplified by arbitrary primers are used as genetic markers. Nucleic Acids Research. 18**(22):**6231-6535.