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Research Article

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Determination of Genetic Diversity in Ornamental Gold Fish Varieties Using RAPD Molecular Markers

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ABSTRACT

The objective of this study was to find out the genetic relationship and variations among different varieties of gold fishes using Random Amplified Polymorphic DNA (RAPD) amplification assay. High degree of polymorphism was observed among the samples, suggesting the degree of genetic variability. The low levels of genetic variation within-species are may be due to their limited migration and pair fidelity mode of reproduction. The dendrogram obtained from RAPD by using two different primers OPA 11 and OPD 8 clearly depicts the genetic similarity and genetic variation within the gold fish species. The data collected from this study will help in understanding their complex traits such as shape, size, colour, morphological variations, tail and life span.

Keywords: *Gold fish, RAPD, PCR, Genetic variations, Polymorphism, Dendrogram, Marker.*

INTRODUCTION

Goldfish, *Carassius auratus* is primarily a freshwater fish regarded to be one of the most popular pet fishes of the world. It is widely distributed in the Eurasian continents and belongs to family Cyprinidae of order Cypriniformes. *C. auratus* is an exceedingly variable organism which includes a range of morphological varieties. Phenotypic traits in goldfish have evolved together with human culture owing to rigorous artificial breeding. All goldfish varieties have originated from a single *Carassius auratus* gibelio ancestor commonly found in Chinese waters[1]. Currently, there are about 300 breeds recognized in China. Goldfish breeds vary greatly in size, body shape, fin configuration and coloration[2].

Some of the main varieties are: Common Gold Fish, Black Moor Gold Fish, Bubble Eye Gold Fish, Celestial Eye Gold Fish, Comet Gold Fish, Fantailed Gold Fish, Pearl Scale Gold Fish, Oranda Gold Fish, Lion Head Gold Fish, Ryukin Gold Fish, Shubunkin Gold Fish, Panda Moor Gold Fish, Ranchu Gold Fish, Telescope Eye Gold Fish, Veiltail Gold Fish, Butterfly Tail Gold Fish, Egg Fish Gold Fish, Lionchu Gold Fish etc.

The diverse family *Cyprinidae*, the most species-rich family of all vertebrates, has been paid only limited attention in population genetics studies. The family *Cyprinidae* with about 2,000 species seems to have originated in Southeast Asia but now is also distributed in fresh water lakes, rivers and streams of Europe, Asia, Africa and North America. About half of the known species are native to Asia [3].

Classification of goldfish family has been based principally on external morphological characters. However, characteristics are apparently influenced by a variety of habitats and environmental conditions. Thus, two sympatric species may be morphologically similar but misidentified as a single species. The basic information on numbers of species or population in a particular area is important for conservation programs. The genetic variety of gold fish in India has not been studied extensively so far by using RAPD fingerprinting. Therefore, this experiment was focused on detection of RAPD pattern for gold fish and determination of the genetic variation among gold fish populations.

Within the last decade, technological advancement has increasingly supported the use of molecular techniques in determining population diversity[4]. Several molecular techniques for evaluating genetic variability in fish species are available which allow ecologists and biologists to determine the genetic architecture of a wide variety of closely related individuals[5,6,7,8,9,10,11]. One such technique is the Random amplified polymorphic DNA (RAPD) which was first introduced by[12]. The characters assessed through RAPD are useful for genetic studies because they provide various types of data-taxonomic population, inheritance pattern of various organisms including fishes[13,14,15]. RAPD reactions are PCR reactions, but they amplify segments of DNA which are essentially unknown to the scientist[16,17]. PCR leads to the amplification of a particular segment of DNA segment. Whereas, in RAPD analysis, the target sequence to be amplified is unknown. The scientist will design a primer with an arbitrary sequence, simply makes up a 10 base pair sequence (or may have a computer randomly generate a 10 bp sequence), then synthesizes the primer[18,19]. The scientist then carries out a PCR reaction and runs an agarose gel to see if any DNA segments were amplified in the presence of the arbitrary primer.

We chose RAPD analysis for this study because it is a simple and rapid method for determining genetic diversity in various organisms with the advantage that no prior knowledge of the genome under study is needed or the polymorphic function as genetic markers.

MATERIALS AND METHODS

Sample collection

7 different varieties of gold fish namely 1.Common Gold Fish, 2.Black Moor Gold Fish, 3.Brass Gold Fish, 4.Veil Tail Gold Fish, 5.Red Cap Oranda Gold Fish, 6.Shubunkin Gold Fish and 7.Pearl Scale Gold Fish were collected from an aquarium shop at Russel Market, Shivajinagar, Bangalore, India. They were brought to the laboratory in a plastic bag containing fresh water with continuous aeration and maintained in glass tank of fresh water. The water was aerated continuously and changed every day. These fishes were fed with fish feed regularly and were acclimatized for 2-3 d in prevailing room temperature. Only healthy fishes were used for experimental analysis.

Genomic DNA Extraction

The DNA was isolated and extracted according to the procedure described by[20]. Approximately 1 cm2 of fresh fish tissues were minced and homogenized with 700µl of TNES (Tris, NaCl, ethylene-di-amine-tetra-acetic-acid and sodium dodecyl sulphate), Urea Buffer (10 mM Tris pH 7.5, 125 mM NaCl, 10 mM ethylene-diamine-tetra-acetic acid (EDTA), 0.5% sodium dodecyl sulphate (SDS) and 4M urea) and 50µg/ml of proteinase K followed by overnight incubation at 37ºC. The digested tissues were then subjected to RNase treatment to remove the RNA present in sample. 20 µg/mL concentration of RNase solution were added to the reaction tubes and the samples were incubated at 37ºC for 1 hour. The supernatant were then treated with an equal volume of phenol/chloroform/isoamyl alcohol (25: 24: 1) three times followed by centrifugation at 10,000 rpm for 10 minutes. The supernatant was extracted with equal volume of chloroform/isoamyl alcohol (24: 1) and centrifuged at 10,000rpm for 10 minutes. The DNA was finally ethanol (95%) precipitated, washed with 70% alcohol, air-dried and resuspended in 50µl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) and stored at 4º C for further use.

Qualitative and Quantitative estimation of DNA

DNA quality was assessed according to[21] by using Nanodrop Spectrophotometer (Thermoscientific Nanodrop 1000, USA) at the absorbance ratio of 260 and 280 nm providing a value of 1.8 which determines pure DNA preparation.

The concentration of DNA in the sample was calculated using the given formula:

Concentration of DNA = A260× 50 µg× dilution factor

Purity of the DNA = A260: A280 ratio = A260 /A280

Quality of DNA fragment was electrophoretically analyzed through 0.8% agarose gel using 1X TAE buffer at 50 V for 45 mints. A 500 base pair ladder (Chromus Biotech) was loaded into the gel as molecular size marker. The gel was visualized by staining with Ethidium bromide (1µl/10ml) and the bands were seen under UV light by using gel documentation system alpha imager hp (Innotech, USA).

RAPD PCR amplification

RAPD PCR amplification was performed to amplify randomly unknown target sequences by using arbitary random primers according to the protocol described by [22]. PCR was carried out in a 25µl reaction volume containing 2.5µl of 10X Taq buffer, 1.5µl of dNTPs, 1µl of each primer, 1µl of Taq polymerase, 18 µl of water and 1µl of DNA (100 ng/ µl) for each sample in a Corbett Research CG1- 96 PCR Palm Cycler. OPA-11(CAATCGCCGT) and OPD-8 (GTGTGCCCCA) primers were used for RAPD amplification. The primers were purchased from Operon Technologies, USA. The thermal cycle profiles for 36 cycles were as follows denaturation at 94ºC for 1 min, annealing at 37ºC for 1 min, extension at 72ºC for 1 min. There was also an initial denaturation step for 5 min at 94ºC and, at the end of the 30 cycles, a final extension at 72ºC for 10 min and finally hold at 4ºC.

Separation and visualization of amplified products

After amplification, PCR products were electrophoretically analyzed through 1.5% agarose gels, in 1X TBE buffer in a Protean II xi Cell (Bio-Rad, USA) Electrophoresis unit at 100 V for 90 minutes. l0 µl (6 µl of amplified sample and 4 µl of tracking dye) of sample was loaded into each well of the 1.0 mm thick gels.

Gels were stained with ethidium bromide and photographed under UV light by using gel documentation system alpha imager. The sizes of the amplified products were determined by comparison with a 500 bp ladder.

Analysis of RAPD data

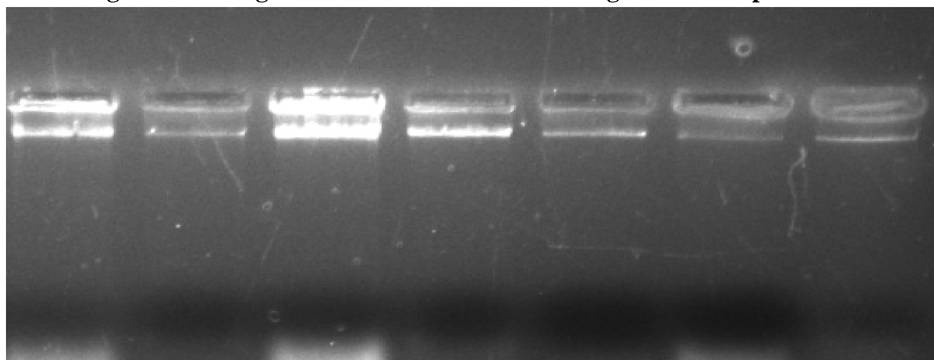
Data on the presence or absence of RAPD bands of identical molecular sizes were used for estimating genetic similarity coefficients. For all pairwise combinations, genetic similarity indices (SI) were calculated following the method of [23]. The formula for SI is given as $SI = 2.N_{AB}/(N_A+N_B)$, where N* is the number of RAPD bands shared in common between individuals A and B, and N_A and N_B are the total number of bands scored in A and B, respectively. The similarity matrix is calculated by Frequency similarity Index obtained from alpha imager hp gel doc software. Dendrogram for RAPD fragments were constructed by using an un-weighted pair group method of arithmetic mean of UPGMA[24].

RESULTS AND DISCUSSIONS

Qualitative estimation of DNA by Agarose gel Electrophoresis

The quality of DNA extracted from different gold fish samples were analyzed by staining with Ethidium bromide and the bands were seen under UV light by using gel documentation system alpha imager hp (Innotech, USA). The single sharp bands in all the 7 lanes clearly indicated the presence of DNA in all samples without any RNA contamination (Figure 1).

Fig.1: Isolated genomic DNA bands of various gold fish samples in the order



1. Common Goldfish, 2. Brass Goldfish, 3. Black moor goldfish, 4. Shubunkin Goldfish, 5. Pearl Scale Goldfish, 6.Veil Goldfish, 7. Red Cap Oranda Goldfish.

Quantitative estimation of DNA by Nanodrop Spectrophotometer

Spectrophotometric analysis of the DNA samples showed the concentration of DNA obtained from Goldfish samples in ng/µl were found to be 1865.6, 2136.2, 1561.9, 2402.1, 3408.9 and 1661.4 and 2125.8 respectively. The 260/280 ratio of the samples obtained were 1.86, 1.81, 1.99, 1.88, 1.88, 1.86 and 1.83 respectively which indicates the presence of pure DNA.

RAPD amplification

The RAPD profile of bands obtained in the 7 varieties of gold fish populations with the two primers OPA-11 and OPD-8 are shown in the Figures 3 and 4 as representative photographs. Number1-7 represented as different samples and M is the molecular marker (500 bp) of low range DNA ladder.

These two primers generated a total of 46 bands in all the 7 individuals out of which 61.85 % were polymorphic, 31.35% monomorphic and 6.39% unique.

Table-1: Pattern of polymorphism and uniqueness (2 primers) between 7 individuals of Goldfish

Pattern of polymorphism	OPA-11	OPD-8	Total
Total No. of bands	17	29	46
Total No. of polymorphic bands	10	19	29
Total No. of monomorphic bands	6	8	14
Total No. of unique bands	1	2	3
Polymorphism, %	58.2	65.5	61.85
Monomorphism, %	35.2	27.5	31.35
Uniqueness, %	5.88	6.89	6.385

Fig.2: Random amplified polymorphic DNA fragment patterns generated using OPA-11 Primer

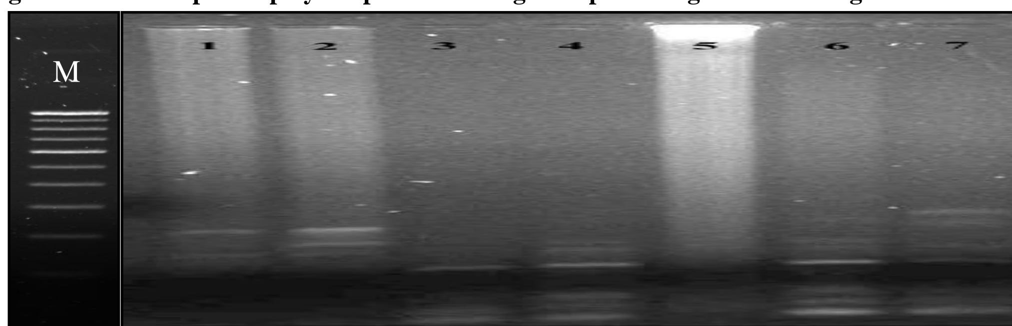


Fig.3: Random amplified polymorphic DNA fragment patterns generated using OPD-8 Primer

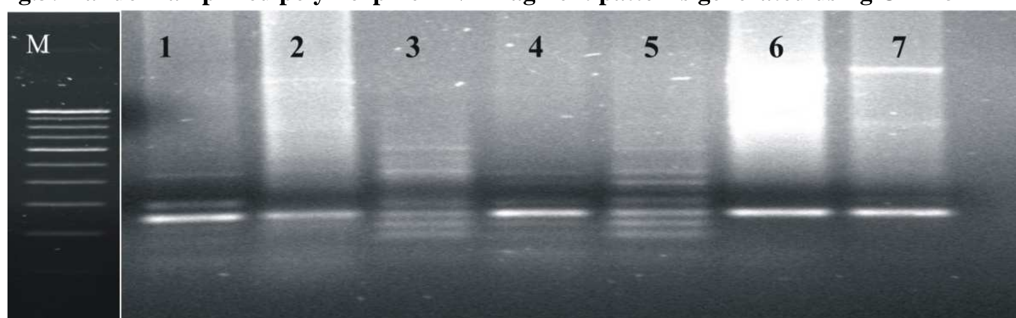


Fig.4: Dendrogram constructed by Similarity Matrix by using OPA-11 Primer

Fig.5: Dendrogram constructed by Similarity Matrix by using OPD-8 Primer

The UPGMA dendrogram was prepared based similarity matrix indicating the segregation of the goldfish populations collected from Bangalore, India. Dendrogram obtained from OPA-11 Primer RAPD data showed that the sample no 1(Common Goldfish) was closely related to sample no 5(Pearl Scale Goldfish) and sample no 4 (Shubunkin Goldfish) was closely similar to sample no 6 (Veil Goldfish). In the other hand dendrogram obtained from OPD-8 Primer RAPD data showed that sample no 1(Common Goldfish) and Sample no 4. (Shubunkin Goldfish), sample no 3 (Black moor goldfish) and sample no 6 (Veil Goldfish) showed maximum similarity to each other.

RAPD-PCR was used here to find out the genetic variability of 7 different goldfish samples (*Carassius auratus*). This technique is based on the detection of polymorphisms, bands observed in some individuals but absent in others, which are amplified by random primers during the PCR. In this study, a high degree of polymorphism was observed, suggesting a high degree of genetic variability between the samples. RAPD markers have been found to have a wide range of applications in gene mapping, population genetics, molecular evolutionary genetics and plant and animal breeding [25, 26]. The RAPD technique consists of amplification by PCR of random segments of genomic DNA using a single-short primer of arbitrary sequence. There is no requirement of prior knowledge of the sequence of DNA. Its cost effectiveness provides an advantage in popula-tion genetic studies. RAPD technique has been applied to the study of phylogentic relationship in tilapia and cichlid species [27]. The presence of variability among populations as well as individuals within a population is essential for their ability to survive and successfully respond to environmental changes [28]. The goldfishes are commercially important fishery species in central India. The genetic stock of gold fish has not been studied in the reservoirs in India. In the present investigation, RAPD analysis has been used to discriminate between the different populations of gold fish. RAPD fragments observed in the 7 individuals, showed a reasonable degree of genetic diversity within and between the populations. The percentages of polymor-phism, monomorphism and uniqueness were 61.85, 31.35 and 6.39, respectively in all the bands obtained from the 2 primers. The population specific bands could not be discerned from the fragment patterns generated.

Our results illustrate that RAPD analysis is a rapid and convenient technique to generate useful genetic markers in goldfishes. The low levels of within-species genetic variation exhibited in are due to their limited migration and pair fidelity mode of reproduction. Similar observations were reported by[9] in carp species. RAPD technique can be use as a rapid method for developing genetic molecular markers for gold fishes. Random primer OPA 11 and OPD8 seems to be good candidate for developing markers. Species-diagnostic RAPD markers should be developed which can be utilized to determine from generation to generation a comparison of growth performance of each gold fish species under commercial growing conditions.

CONCLUSION

The effectiveness of RAPD in detecting polymorphism among different gold fish varieties, their applicability in population studies, and the establishment of genetic relationships among various gold fish populations has been investigated this study. The result obtained from this study has proven to be useful in discrimination, characterization and differentiation of the goldfish and clustering them according to

their origin However, it is essential to optimize RAPD amplification condition and ascertain the reproducibility of RAPD markers for individual taxa prior to apply RAPD fingerprinting to any genetic analysis. By using two different primers, RAPD fragments showed a reasonable degree of genetic similarity as well as variation within and between the species. This observation clearly depicted the genetic similarity and genetic variation within the gold fish species. The genetic data collected during this work will guide the choice of genotypes to cross according to their lineage belonging or their level of diversity.

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