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Assessment of genetic diversity of *Clarias batrachus* using RAPD markers in three water bodies of Bhopal

R.K. Garg, P. Sairkar, N. Silawat N. Batav and N.N. Mehrotra*

Centre of Excellence in Biotechnology, M.P. Council of Science and Technology (MPCST)
Vigyan Bhawan, Nehru Nagar, Bhopal - 462 003, India
*Central Drug Research Institute, Lucknow - 226 001, India

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Abstract: *To assess the extent of genetic diversity within and between three populations of the catfish, Clarias batrachus, an analysis for random amplified polymorphic DNA (RAPD) variations were carried out. Five out of ten random primers produced constant and reproducible pattern of RAPD products. These five primers produced 72 scorable DNA fragments out of which 68 (86.66%) were polymorphic. Populations showed significant differences in their degree of polymorphism. Differences however, were observed in the primers in producing similarities in the populations.*

Key words: *Catfish, Genetic diversity, Similarity coefficient, RAPD, Polymorphism*
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Introduction

Clarias batrachus popularly known as magur is an air breathing fish and well adapted to adverse ecological conditions. They normally inhabit in swamps, marshy and derelict waters. These water bodies are usually shallow with heavy silt of decaying vegetation and organic load with poor nutrient release. Besides, these water bodies have low pH, oxygen and primary productivity, high carbon dioxide, hydrogen sulphide, methane and free ammonia, and this type of adverse environment is quite insensitive to the above air breathing slow growing, hardy omnivorous fishes (CAFF, 2006). However, their population/density has decreased significantly during last two decades, owing mainly to imprudent development and reckless fishing. Therefore, there is a need to understand the genetic composition of natural population of *Clarias batrachus* in order to evaluate the latent genetic effects induced by hatchery operations.

Molecular markers are realistic and useful tools for the investigation and monitoring of genetic conditions both in native populations and in captive lots (Alam and Islam, 2005). RAPD (Random Amplified Polymorphic DNA) and microsatellite markers are among the molecular markers used to analyze genetic diversity of fish. Both of these markers may be analyzed by PCR (Polymerase Chain Reaction). A large number of potential markers can be generated using readily available markers. This, in addition to its swift pace of obtaining data, makes RAPD a popular technique, which has been used in studies of inter-specific hybridization, introgression, identification of clones, development of markers linked to sex-determination and measurements of genetic diversity, as well as many other studies (Wolfe and Liston, 1998). The control of gene expression has been a focus of molecular biology since classic experiments in the 1950's first began to explore how DNA fulfilled its heredity role. New findings are now demonstrating that low level exposures to a variety of agents including environmental agents/contaminants can alter gene expression (Yoon *et al.,* 2008).

Inspite of its economic and scientific importance, little information is available on the phylogenetic relationship among the few catfish populations in Madhya Pradesh. RAPD technique is also one of the most frequently used molecular methods for taxonomic and systematic analyses of various organisms (Bartish *et al.,* 2000, Garg *et al.*, 2009a). Therefore, the objective of the present work is to identify genetic similarity and diversity within and between the populations of *Clarias batrachus* from three water bodies of Bhopal as revealed by RAPD-PCR.

Materials and Methods

Three water bodies of Bhopal *i.e.*, Upper lake, Lower lake and Shahpura lake were selected for detailed study of intra-specific genetic diversity of *Clarias batrachus*. Specimens were collected with the help of local fishermen. All the fishes were brought to the Biotechnology Core Laboratory, MPCST on the same day. The morphometric measurements of all the fishes were done. Three individuals from each water body were caught for the morphometric measurements and total 15 parameters *i.e.*, total length, standard length, body weight, body width, body depth/height, body length, head length excluding snout, width of head, snout length, eye diameter, length of caudal peduncle, length of anal fin, length of pelvic fin, length of pectoral fin, height of caudal peduncle were taken mean and standard deviation (SD) were calculated to obtained average value of all morphometric parameter. Fish specimens were morphologically

* **Corresponding author:** rkgargmpcst@gmail.com

Table - 1: Morphometric measurement of *Clarias batrachus* collected from three water bodies of Bhopal

Measurement parameter	Upper lake			Mean±SD	Lower lake			Mean±SD	Shahpura lake			Mean±SD
	UL1B	UL2A	UL3A		LL1A	LL2A	LL2B		SL1A	SL2B	SL3A	
Total length	12.50	15.50	18.00	15.33±2.753	19.00	11.50	12.00	14.160±4.193	38.00	20.00	35.00	31.00±9.60
Standard length	11.40	14.00	16.50	13.96±2.550	16.00	10.50	11.00	12.50±3.041	33.00	18.00	20.00	23.60±8.10
Length of head	3.00	2.00	5.00	3.33±1.527	7.00	2.50	3.70	4.40±2.330	8.00	6.00	8.20	22.20±1.21
Height	2.00	1.80	2.50	2.10±0.360	3.50	1.30	2.20	2.33±1.106	3.50	4.00	3.00	3.50±0.50
Length of caudal peduncle	1.50	1.50	3.00	2.00±0.866	2.50	1.00	1.50	1.66±0.763	2.00	3.50	2.50	2.60±0.28
Height of caudal peduncle	1.70	1.00	2.60	1.76±0.802	2.50	1.00	1.50	1.66±0.763	2.50	2.50	3.00	2.60±0.75
Length of the head excluding snout	3.00	2.70	4.30	3.33±0.850	5.50	4.40	2.00	3.96±1.789	5.00	0.20	3.50	2.90±0.10
Diameter of eyes	0.30	0.50	0.30	0.36±0.115	0.50	0.20	0.30	0.33±0.152	0.80	0.60	0.70	0.70±4.40
Snout	0.50	1.00	0.60	0.70±0.264	0.30	0.40	0.80	0.50±0.264	11.00	2.50	9.20	7.50±0.65
Pre-dorsal length	3.50	3.50	5.30	4.10±1.039	6.60	4.00	2.50	4.36±2.074	5.50	5.00	4.40	4.90±0.28
Width of head	2.00	2.00	3.00	2.33±0.577	2.50	2.40	2.30	2.40±0.100	4.20	3.70	4.00	3.90±0.142
Weight, gm	11.00	19.15	25.25	18.46±7.149	210.00	185.00	1800	211.60±32.885	220.00	190.00	210.00	206.60±32.885

All values in cm otherwise stated

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identified with taxonomic keys (Shrivastava, 2000; Jayaram, 1999). The muscle, liver and brain tissues were isolated from freshly caught fishes for molecular studies.

For the isolation of total genomic DNA, a short procedure was applied according to a modified protocol than reported (Wu *et al.,* 1995). Liver tissues (200-500 mg) were placed in a 1.5 ml micro-centrifuge tube and homogenized by using Eppendorf micro-pestle. In the homogenized tissue, 0.5 ml of lysis buffer (4 mM NaCl, 0.5 mM EDTA, 0.1% SDS and 0.02 NP 40) and 0.01% proteinase-K were added, mixed gently and incubated at 55°C on dry bath (Genei, Model-SLM-DB-120) for 45-60 minutes for complete lysis of cells. After incubation, 250 µl chloroform and 250 µl phenol were added, mixed gently, and centrifuged at 10,000 rpm at room temperature (High Speed Brushless Centrifuge, MPW-350R) for 5 minutes. The supernatant was then transferred to a new micro-centrifuge tube and further chloroform (250 µl) and 250 µl of 7.5 M ammonium acetate were added, mixed well and centrifuged at 10,000 rpm for 5 minutes at room temperature. The DNA was precipitated from supernatant with two volumes of 99% ethanol (ice-cold). The DNA pellet was then washed with 70% ethanol, dried and dissolved in a Tris-EDTA buffer (10mM Tris HCl, 1mM EDTA, pH 7.6). UV-VIS Spectrophotometer (ND-1000, USA) was used to check quality as well as quantity of isolated DNA. The concentration of extracted DNA was adjusted to 50 ng μl^{-1} for PCR amplification.

In this work, ten commercially available oligonucleotide primers (ten to twenty bases long) of Bangalore Genei, India were used to initiate PCR amplifications. Primers were randomly selected on the basis of GC content and annealing temperature for RAPD-PCR amplifications. After initial screening with all the ten primers, the five which were used in final study were RAn-1, RAn-3, RAn-4, RAn-5 and RAn-6.

The reaction mixture (30 µl) for PCR was composed of 3 µl of 10X Taq polymerase buffer, 1.2 µl of 10mM dNTP, 1.2 µl of RAPD primer, 0.6 µl Taq DNA polymerase (3U μl^{-1}), 23.4 µl sterile distilled water and 0.6 µl template DNA. A negative control, without template DNA was included in each round of reactions. After pre-heating for 5 minutes at 94°C, PCR was run for 45 cycles. It consisted of a 94°C denaturation step (0.45 minutes), 37°C annealing step (1 minute) and 72°C elongation step (1.5 minutes) in a Thermal Cycler (Corbet Research, Australia). At the end of the run, a final extension period was appended (72°C, 7 minutes) and then stored at 4°C until the PCR products were analyzed.

The amplified DNA fragments were separated on 1.2% agarose gel and stained with ethidium bromide. A DNA size marker was run with each gel (100, 200, 300, 600, 1000, 1500, 2000, 2500 and 3000 bp DNA ladder from Bangalore Genei, Bangalore, India). The amplified pattern was visualized on an UV transilluminator and photographed by Gel Documentation System (Alpha-Innotech, USA). The RAPD fragments were scored for the presence and absence of fragments on the gel photographs, and RAPD fragments were compared among the populations. RAPD banding patterns were recorded on spreadsheets, which were used to determine gene diversity, gene flow, number of polymorphic loci and genetic distance through a construct by an un-weighted pair group method of arithmetic mean (UPGMA) as per Nei, 1978.

Results and Discussion

Most of the morphometric characteristics of fishes are similar and often overlap with population (Table 1). These morphometric

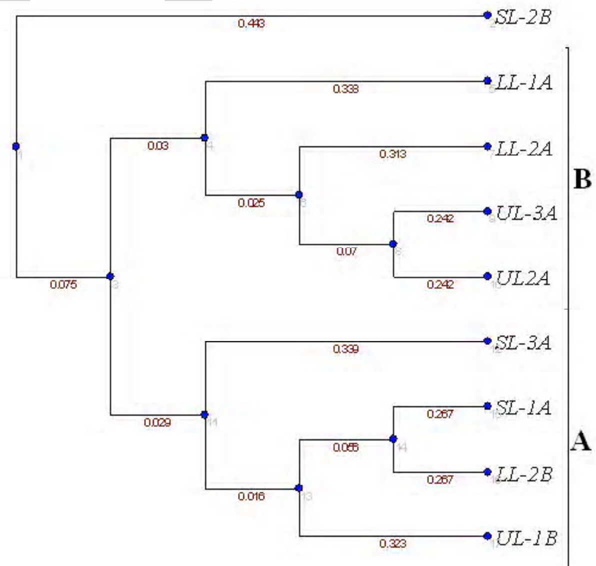

Fig. 1: A dendrogram based on UPGMA clustering of 09 individuals using genetic distance

Table - 2: Jaccard's similarity index between different individuals of *Clarias batrachus*

Sample code	UL-1B	UL-2A	UL-3A	LL-1A	LL-2A	LL-2B	SL-1A	SL-2B	SL-3A
UL-1B	1.00								
UL-2A	0.405	1.00							
UL-3A	0.282	0.515	1.00						
LL-1A	0.238	0.333	0.315	1.00					
LL-2A	0.400	0.361	0.424	0.256	1.00				
LL-2B	0.368	0.333	0.219	0.181	0.40	1.00			
SL-1A	0.375	0.294	0.235	0.189	0.281	0.466	1.00		
SL-2B	0.125	0.090	0.030	0.058	0.064	0.241	0.217	1.00	
SL-3A	0.303	0.228	0.242	0.228	0.250	0.264	0.400	0.080	1.00

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Tabl e -3: Cluster composition of 09 individuals of *Clarias batrachus* based on RAPD markers

Cluster	Serial number of individuals	Origin
A	SL-3A, SL-1A, LL-2B, UL-1B	Shahpura lake, Lower lake, Upper lake
B	LL-1A, LL-2A, UL-3A, UL-2A	Lower lake, Upper lake
C	SL-2B	Shahpura lake

data observed in *clarias batrachus* from three water bodies are insignificant to support the established genetic structure of the population that often leads to taxonomic uncertainty (Daniel, 1997; Ponnian and Gopalakrishnana, 2000; Garg *et al.*, 2009b).

The isolation of high quality DNA is essential for many molecular biology applications including polymerase chain reaction (Chakraborty *et al.,* 2008). Isolated DNA was qualitatively and quantitatively estimated by Nano-Drop UV-Spectrophotometer (ND-1000, USA). The purity of DNA sample was also calculated from 260/280 OD ratios (Sambrook and Russell, 2001). After checking the quality and quantity of isolated DNA, initially ten random primers (animal primers) were screened. Only the primers which displayed reproducible, scorable and clear bands were considered for analysis. The image profiles of banding patterns were recorded and molecular weight of each band was determined by Alpha-view software. The banding pattern was scored on the basis of presence or absence of clear, visible and reproducible bands. The results were analyzed based on the principle that a band is considered to be 'polymorphic' if it is present in some individuals and absent in others. The RAPD fragments were scored for the presence and absence of fragments on the gel photographs and RAPD fragments were compared among the samples collected from different lakes. RAPD banding patterns were recorded on spreadsheets, which were used to determine Nei's (1978) gene diversity, gene flow, number of polymorphic loci, genetic distance and Jaccards similarity coefficient (Table 2) and the construct an un-weighted pair group method of arithmetic mean (UPGMA) dendrogram among the samples collected from different places using Cluster-vis-software.

Of 10 decamer primers used to screen representative DNA samples, 5 (50%) detected scorable polymorphism in banding pattern among all the 09 individuals. Five selected primers generated a total of 72 bands of which 68 were polymorphic. An example of the representative profiles of 09 individuals with two primers is shown in Fig. 1. The number of bands per individuals ranged from 01 to 10 and bands amplified ranged in size from 172 to 1677 bp. The average number of bands per primer ranged between 06 (RAn-4) and 21 (RAn-3) with a mean of 14.40. The proportion of polymorphic markers across the primers ranged between 33.33 and 100% with an average of 86.66% (Table 4). Shifat *et al.* (2003) have also reported RAPD Jaccards dissimilarity coefficient in 34 individuals of *Tenualosa ilisha* and observed 20.41% polymorphism. Rahman *et al.* (2009) studied genetic variations of wild and hatchery populations of *Catla catla* revealed by RAPD markers and found overall 54.55% polymorphism. We found that 86.66% of the loci in our study were polymorphic as compared to the 75% reported by Islam *et al.* (2005) in *Catla catla*, 55.76% in *Oreochromis niloticus* (Zaeem and Ahmed, 2006) and 64.98% in *Mystus vittatus* by Garg *et al.*, 2009c.

UPGMA dendrogram revealed that one of the individual SL-2B from Shahpura lake was monotypic and displayed maximum dissimilarity coefficient of 0.443 with other individuals, while, other eight individuals clearly distributed in two clusters (Fig. 1). The distribution of different individuals revealed that there were four individuals in cluster I *i.e.*, SL-3A, SL-1A, LL-2B, UL-1B and four in cluster II *i.e.*, LL-1A, LL-2A, UL3A and UL-2A (Table 3).

The RAPD analysis proved to be an effective and efficacious technique to measure the magnitude of diversity and discriminate between genotypes, as against the insufficient morphometric parameters.

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Table - 4: Comparison of genetic diversity among 9 individuals of *Clarias batrachus*

Primer number	Accession number	Total no. of bands	Polymorphic bands	% of polymorphism	Molecular weight range (kb)
RAn-1	AM765825	14	14	100.0	172-1351
RAn-3	AM765834	21	21	100.0	600-1280
RAn-4	AM750059	06	02	33.33	306-1050
RAn-5	AM750052	15	15	100.0	600-1280
RAn-6	AM765829	16	16	100.0	188-1329
Total score		**72**	**68**	**-**	**-**
Mean per primer		**14.40**	**13.60**	**86.66**	**-**

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