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RAPD-based genotyping of walking catfish, *Clarias batrachus* in a population of Bangladesh

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Abstract

Genetic diversity of any fish is most important for their existing, breeding, production and management. In this study, intra-species genetic variation of a wild population of walking catfish, *Clarias batrachus* in Sylhet, Bangladesh was investigated. Four RAPD markers such as OPB-19, OPB-12, OPB-05 and OPF-14 were used to assess genetic variability among 8 individuals of *C. batrachus* genotypes. Less number of DNA bands was revealed by all the primers in experimental populations where a total of 132 bands were detected and all the markers were shown different levels of polymorphism. Intra-specific polymorphisms were recorded 60%, 55.56%, 40.91% and 30.77% in primer OPB-12, OPB-19, OPF-14 and OPB-05 respectively where average polymorphism was recorded 46.81%. Average gene diversity was found 0.19823. The genetic distance of experimental samples ranged from 0.6 to 1 and in an average 0.88, indicating higher genetic distances of this population. A genetic relationship among the individuals of the experimental population was constructed where 3 clades and 2 sub-clusters were recorded considering different linkage distances. Considering different genetic parameters genetic variability was found higher among this experimental population.

Keywords: Genetic diversity, walking catfish, RAPD, *Clarias batrachus*

Introduction

Bangladesh is rich in fish diversity in both marine and freshwater environments whereas interestingly Bangladesh is third position in fisheries production^[1]. The fisheries sector plays a vital role in Bangladesh in terms of the economy where this sector contributes 4.64% of GDP^[2]. Among different economically valued fishes of Bangladesh, walking catfish *Clarias batrachus* is one of the popular and highly nutritious, belonging to the order Siluriformes^[3]. It is recognized as traditional food for pregnant and lactating mothers as well as elders and childrens due to its low-fat content and medicinal characteristics^[4]. This fish is particularly well famous and cultured extensively in several countries of South Asia and South East Asia including India, Bangladesh, Pakistan, Myanmar, Thailand, Vietnam, etc.^[5]. This fish is very easy to culture even in harsh environments and it can be stored in a container with water without giving any food for days by modifying accessory air-breathing organ. This walking catfish is widely distributed in different freshwater bodies such as ponds, haors, baors, beel, jheel and rivers of Bangladesh^[5]. It was once easily available in almost all freshwater sources in Bangladesh but now it is endangered because of extensive catching, adverse changes to nature due to human aggression and the destruction of its natural habitats^[6]. Therefore, the enhancement of natural stock is the most important of this fish in Bangladesh. Though induced breeding was successfully done, however, available fry of this walking catfish is still the main obstacle for farming of *C. batrachus*^[7-8]. The quality of this fish is also needed to improve as this fish is used in different important purposes.

Expansion, improvement of natural stock and maintaining gene pool in any species is essential for adaptation in different conditions whereas genetic variation is one of the important key tools for assessing the above biological parameters of an organism. Though several researches have been conducted considering different aspects of this fish, however, limited genetic researches have been conducted^[4, 9-12] and very limited genetic study has also been performed in Bangladesh^[9]. Though different molecular approaches including genome sequencing for genetic characterization of this fish were recorded^[12], however, RAPD (Random Amplified Polymorphic DNA) technique is suitable for knowing genetic status of this fish considering genetic variation, the relationship among individuals and between populations and

development of an organism and so on. Therefore, in this study, a cost-effective and simple RAPD technique was analyzed to assess the genetic diversity in a wild population of *C. batrachus* in Bangladesh.

2. Materials and Methods

2.1. Sample collection

The experimental samples of fish samples were collected by traditional fish catcher from haor habitat (a type of freshwater) of Sylhet, Bangladesh, which were then brought to the Fisheries and Marine Biotechnology Research Unit in the Department of Genetic Engineering and Biotechnology (GEB) at Shahajalal University of Science and Technology (SUST), Sylhet, Bangladesh. The collected fish samples were identified through morphometric characteristics of Shafi and Quddus (1982) [3] and Rahman (1989) [5] and kept in aquariums by maintaining the proper environment until tissue isolation.

2.2. DNA Extraction

The fishes were dissected to take out the liver and kidney by using scissors, forceps and needles. The isolated tissues of the fishes were taken separately into the petridish and washed with distilled water. Then the organs were kept in falcon tubes using 70% ethanol and preserved them at -20°C until DNA extraction. DNA extraction was done from tissues using a commercially available kit, Gene JET and the quality of extracted DNA was checked by gel electrophoresis using 0.8% agarose. After that, the DNA samples were stored at -20°C.

2.3. PCR amplification

Genotyping of Asian catfish, *C. batrachus* in a population of Bangladesh was studied using four decamer primers and the selected primers were OPB-19, OPB-12, OPB-05 and OPF-14. PCR reaction was carried out using GoTaq® G2 Hot Start Green Master Mix and 15 µl PCR mixtures were used for each DNA sample. PCR reaction was conducted with pre-heating at 94 °C for 3 minutes followed by 35 cycles of denaturation at 94 °C for 1 minute; annealing temperature for selected four primers OPB-19, OPB-12, OPB-05 and OPF-14 were 29 °C, 27 °C, 29 °C and 30°C respectively for 1 minute and 2 minutes elongation or extension at 72 °C. A final step of 7 min for 72°C was added to allow complete extension of the amplified fragments. The amplified PCR products from each sample were checked for the banding pattern of DNA by electrophoresis on 1.2% agarose and gel DNA was contained using ethidium bromide. A ladder (1kb Gene Rullar, USA) ranging 250bp-10000bp was used for comparison of DNA quality. The agarose gel of the amplified PCR products was placed on the gel documentation to take the photograph (Panasonic™ DMC-FS20, 10 Megapixels).

2.4. Assessment of genetic variability

RAPD data of this experiment was interpreted by using

different software and equations whereas Alpha Ease FC 4.0 was used for the identification of bands at molecular level. Polymorphism information content (PIC) was measured using a formula where the PIC is $2f_i(1-f_i)$ whereas f_i is the frequency of the amplified allele (band present). The average number of alleles per locus was calculated by a formula and the formula is

$$N = \frac{1}{K} \sum_{i=1}^K n_i$$

Where K is the number of loci and NI is the number of alleles detected per locus. Allele frequency and average gene diversity were calculated according to $(p+q)^2 = p^2+2pq+q^2$ Formula of Hardy- Weinberg equilibrium. Whereas H_j is considered as Intra locus gene diversity $(1-p^2-q^2)$, as well as the average heterozygosity (H_i) was calculated for the population genetic structure of this fish. Genetic distance was measured using the formula of $D = 1 - N_{xy} / (N_x + N_y - N_{xy})$, where D is the genetic distance between sample x and y; N_{xy} is the number of bands shared by sample x and y; N_x is the number of bands in sample x and N_y is the number of bands in sample y. Linkage distance was calculated with the measurement of Squared Euclidean distances and genetic relationships were drawn based on linkage distance.

3. Results

3.1. DNA profile and data scoring

In this study, four decamer primers were used to assess the genetic variability among 8 individuals of *C. batrachus* genotypes. The banding pattern of DNA was checked using 1kb plus DNA ladder (Thermo Scientific Generuler) considering molecular weight ranges from 75 bp to 10,000 bp (Generuler™). Each amplified band profile was defined by the presence or absence of bands at particular positions on the gel. Fragments were scored as 1 if present or 0 if absent, separately for each individual and each primer. Less number of DNA bands was revealed by all the primers in experimental population where a total of 132 bands were detected among the individuals. All markers were shown at different levels of polymorphism where the highest number of bands (51) amplified by the primer OPB-19 and the lowest number of bands (20) amplified by the primer OPB-05. Polymorphisms were recorded 60%, 55.56%, 40.91% and 30.77% in primer OPB-12, OPB-19, OPF-14 and OPB-05 respectively where average polymorphism was recorded 46.81%. The highest number of bands (6.38) per sample was amplified from the primer OPB-19 and the lowest number of bands (2.5) per sample was amplified by the primer OPB-05. The primer OPB-12 and OPF-14 amplified 3.5 and 4.125 numbers of bands per individual sample respectively. Primer OPB-12 shows the highest polymorphism information content (PIC) (0.334491) while an average PIC was 0.314514 (Table 1).

Table 1: RAPD based analysis experimental individuals of *Clarias batrachus*.

Primer	Size of DNA Bands (bp)	Total Number of DNA Bands	Number of Polymorphic loci	Percentage of Polymorphic loci	Number of Bands per sample	Polymorphism information content (PIC)
OPB 19	232-2806	51	15	55.56	6.38	0.334491
OPB 12	1331-3736	28	9	60	3.5	0.358333
OPB 05	1411-9281	20	4	30.77	2.5	0.274038
OPF 14	530-7561	33	9	40.91	4.13	0.291193
Total		132	37			
Average		33	9.25	46.81	4.13	0.314514

3.2. Allele frequency and average gene diversity

Allele frequency and average gene diversity were calculated according to Hardy-Weinberg equilibrium. Intra locus gene diversity and average gene diversity were recorded 5.81 and

0.215055 in primer OPB-19, 3.43 and 0.228815 in primer OPB-12, 2.26 and 0.173712 in primer OPB-5 and 3.86 and 0.175337 in primer OPF-14 respectively (Table 2).

Table 2: Summary of allele frequency and Intralocus gene diversity for all primers.

Primer	Lowest Allele Frequency		Highest Allele Frequency		Lowest gene diversity	Highest gene diversity	Average gene diversity
	q	p	q	p			
OPB-19	0.707107	0.064586	0.935414	0.292893	0.120829	0.414214	0.215055
OPB-12	0.790569	0.064586	0.935414	0.209431	0.120829	0.331139	0.228815
OPB-05	0.612372	0.064586	0.935414	0.387628	0.120829	0.474745	0.173712
OPF-14	0.790569	0.064586	0.935414	0.209431	0.120829	0.331139	0.175337
Average							0.19823

3.3. Evaluation of genetic distance

In this experiment, the genetic distance of Asian catfish, *C. batrachus* was calculated whereas lowest genetic distance was found 0.6 and highest distance was recorded 1 and average genetic distance was recorded 0.88. It was seen that comparative higher genetic distances were observed in the experimental population (Table 3).

Table 3: Genetic distance among individuals of *Clarias batrachus*

Sample	1	2	3	4	5	6	7	8
1	--	0.6	1	0.9	0.94	1	1	1
2		-	0.91	0.8	0.9	1	1	1
3			-	0.74	0.8	0.91	0.9	1
4				-	1	0.91	1	1
5					-	0.9	0.9	1
6						-	1	1
7							-	0.7
8								-

3.4. Genetic relationships among individuals

A genetic relationship among the individuals of the experimental population was analyzed (Figure 1) whereas three clades were found with individual pairs 1 and 2 with a linkage distance value of 15, individual pair 3 and 4 with a linkage value of 20 and individual pairs 7 and 8 with a distance value of 11 respectively. A sub-cluster was made with clades 1 and 2 in respect of linkage distance around 26.5. Individual 5 directly linked with the first sub-cluster and made the second sub-cluster with the linkage distance around 28.5, and finally, a cluster was formed with sub-cluster 2, clade 1 and individual 6 with linkage distance 30.3.

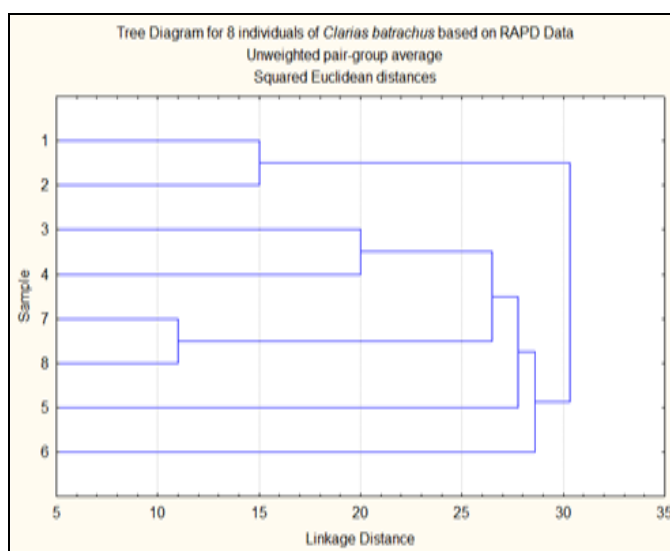


Fig 1: Genetic relationships among individuals of *C. batrachus*

4. Discussion

In this study, intra-species genetic variation of a wild population of walking catfish, *Clarias batrachus* in Sylhet, Bangladesh was investigated using RAPD assay. Less number of DNA bands was revealed by all the primers in experimental populations where a total of 132 bands were detected considering 8 individuals and all the markers were shown different levels of polymorphism, conversely a total of 1376 RAPD bands were revealed in India using five decamer primers considering 16 individuals of *C. batrachus* [13]. The mean intra-specific polymorphism of the experimental population was recorded 46.81% which has been found different in other experiments in India [4, 13]. The present finding was also dissimilar with the finding of Garg *et al.* where they were found a total of 72 bands with 86.66% polymorphism in India using five primers considering 9 individuals [14]. Comparatively lower polymorphism was found in the present study when compared with other research with 6 decamer primers produced 462 bands considering twelve individuals of *C. batrachus* in India but in percent, the present study was found higher polymorphism (46.81) than 35.7 [15]. Average gene diversity was found 0.19823 in the present study and genetic distance of experimental samples ranged from 0.6 to 1 indicating comparative higher genetic distances were observed but this finding was different from the finding of *C. batrachus* by Danish and Singh whereas the genetic diversity was recorded 2.04 [13]. Higher genetic variability among 16 individuals was observed in the hatchery population of walking catfish in India which is similar to the finding of the present study [4]. A genetic relationship among the individuals of the experimental population was recorded with 3 clades and 2 sub-clusters considering different linkage distances whereas associations among the 16 genotypes revealed by UPGMA cluster analysis were found more clusters and clades of *C. batrachus* than the present study [13]. Based on this study considering different genetic parameters higher degree of genetic diversity was found among this experimental population. Genetic diversity of any fish is most important for their existing, breeding and production, etc. However, this experiment would be used for future investigation on this species using different techniques including RAPD RFLP assays with more samples from different habitats for the genetic improvement of this fish.

5. Conclusion

In this experiment, RAPD based genetic variability of wild type *Clarias batrachus* in Sylhet, Bangladesh was analysed. Based on this study considering different genetic parameters higher genetic variability was found in this experimental population. However, it is very much needed for observing

genetic status of this fish with more individuals from different habitats with different markers whereas the genome of this fish is already sequenced^[12]. However, this finding would be used for the future investigation of this species in Bangladesh.

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7. Authors' contribution

All authors contributed equally to the study of this research and during the preparation of the manuscript.

8. Competing interests

The authors declare that they have no competing interests.

9. Consent for publication

All authors approve the manuscript for publication.

10. Reference

1. FAO. The state of world fisheries and aquaculture. FAO. Rome, Italy, 2018, 227.
2. BFS. Bangladesh Fisheries Statistics, 2018.
3. Shafi M, Quddus MMA. Bangladesher Motsyo Sompod. Bangla Academy, Dhaka, 1982, 347.
4. Danish M, Singh IJ, Priyanka Singh GCP. Molecular characterization of two populations of catfish *Clarias batrachus* L. using random amplified polymorphic DNA (RAPD) markers, African Journal of Biotechnology. 2012; 11(77):14217-14226.
5. Rahman AKA. Freshwater fishes of Bangladesh. Zoological Society of Bangladesh, 1989, 364.
6. IUCN. Bangladesher Bipanno Prani, IUCN Bangladesh. The World Conservation Union. Dhaka, Bangladesh. 2000, 294.
7. Ahmed K, Mustafa G, Ali S, Shahjahan M. Induced Spawning of Magur Fish, *Clarias batrachus* (L.) by Stripping Method in Plastic Bowl Hatchery. Bangladesh Journal of Zoology. 1985; 13(1):19-24.
8. Mollah. Domestication of riverine catfish. Report submitted to UGC, 2005, 12.
9. Mohammad NI, Md. Shahidul I, Md. Samsul A. Genetic structure of different populations of walking catfish (*C. batrachus* L.) in Bangladesh. Biochemical Genetics. 2007; 45(9-10):647-662.
10. Debnath Gupta R. Phylogenetic Inference from the Correlation of Some Microsatellite DNA Segments for Indirect Assessment of Genetic Diversity in the Asian Catfish, *C. batrachus*. The ICFAI Journal of Computational Mathematics. 2009; 2(2):30-60.
11. Mohindra V, Tripathi RK, Singh A, Singh RK, Lal KK. Identification of candidate reference genes for quantitative expression analysis by real-time PCR for hypoxic stress in Indian catfish, *Clarias batrachus* (Linnaeus, 1758). International Aquatic Research. 2014; 6(2):61.
12. Li N, Bao L, Zhou T, Yuan Z, Liu S, Dunham R *et al.* Genome sequence of walking catfish (*Clarias batrachus*) provides insights into terrestrial adaptation. BMC Genomics. 2018; 19:952.
13. Danish M, Singh IJ. Assessment of genetic diversity of two populations of catfish *Clarias batrachus* L. using

random amplified polymorphic DNA (RAPD) markers. Journal of Pharmacognosy and Phytochemistry. 2018; 7(3):2345-2352.

14. Garg RK, Sairkar P, Silawat N, Batav N, Mehrotra NN. Assessment of genetic diversity of *Clarias batrachus* using RAPD markers in three water bodies of Bhopal. Journal of Environmental Biology. 2010; 31(5):749-753
15. Gulab DK, Reddy ACS, Mann P, Ravinder K, Muzumdar K. *Clarias batrachus* (Linn.1758) population is lacking genetic diversity in India. Molecular Biology Reports. 2010; 37:1355-1362.