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GENETIC DIVERSITY BY RAPD IN FOUR POPULATIONS OF ROHU *Labeo rohita*

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ABSTRACT

Genetic diversity in four geographically distinct rohu (*Labeo rohita*) populations such as rohu Khulna, rohu India, rohu Faridpur and rohu Barisal was determined by RAPD. The banding pattern showed that rohu India was genetically different from the rest three. A total of 87 bands were found in four rohu populations where 35 bands were polymorphic, indicating 48.38% polymorphisms with an average of 12 bands per primer. The molecular size of amplified DNA fragments ranged from 400 to 1250 bp. Eight unique bands were observed in the four populations of which six were found in rohu Indian population. The genetic distance was highest (0.7221) and genetic identity was lowest (0.4857) between rohu India and rohu Barisal populations. Among three local varieties, the lowest genetic distance and highest genetic identity were found between rohu Khulna and rohu Faridpur populations. The UPGMA dendrogram segregated four populations of rohu into three major clusters - C1, C2 and C3. Rohu India was positioned at a fully different cluster - C3, rohu Khulna was placed in C2 and the rest two populations in C1.

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INTRODUCTION

Labeo rohita (Hamilton, 1822), locally known as rohu, is the most important among the Indian major carp species cultured in Bangladesh, contributing to 10.97% of the total inland production (FRSS, 2016). This species is also one of the most cultured freshwater fish in Bangladesh as well as Sri Lanka, China, Philippines, Malaysia, Nepal and some other countries of Africa (FAO, 2006). In Bangladesh, this fish is considered a delicacy and rich source of protein (Rahman, 2005). *L. rohita* has high market demand and value not only in Bangladesh but also in other countries with production that does not meet the demand. Due to a high market demand, this fish is imported from India and Myanmar (Burma), known as Indian or Burmese rohu in Bangladesh. Import of this fish is not self-dependent and production should be increased. For this purpose an improved breeding program should be undertaken. For successful breeding program a wide range of genetic diversity within population is required (Schierwater et al., 1994). Unfortunately there is a limited amount of data available on the genetic diversity of rohu in Bangladesh. It is known that the geographical distance may create genetic diversity (Dayu et al., 2007). The geographic distances are directly correlated with genetic diversity (Rahman et al.,

2009). In Bangladesh the artificially propagated rohu has been cultured in different geographical regions such as Khulna, Barisal and Faridpur, etc., in different closed ponds. Sometimes these fishes are reared and used as brood fish for breeding program but these fishes may come from the same brood population or same tributary. It is necessary to determine the genetic variability of rohu fish found in different geographical culture areas. In this research three culture areas such as Barisal, Faridpur, Khulna and the imported rohu India were selected to determine the genetic diversity based on RAPD. It is a simple, fast and sensitive technique that can identify genetic variation without prior knowledge of DNA sequences and has been widely used in fisheries studies (Akter et al., 2010) to identify the genetic diversity and conservation of fish populations (Almeida et al., 2001). Thus the present study was conducted to elucidate the genetic diversity among three rohu populations collected from geographically distant locations and one imported from India.

MATERIALS AND METHODS

Sample collection and isolation of total genomic DNA

Caudal fins of artificially propagated 10 rohu fishes of each location were randomly collected from different local markets of Dhaka City such as Swarighat, Aanando Bazar, Polashi Bazar and New Market, and stored in 95% ethanol. Approximately, the mean length and weight of rohu India was 2.90±0.12 kg & 53.9±2.18 cm, rohu Khulna was 1.22±0.18 kg &39.9±1.91 cm, rohu Faridpur was 1.20±0.15 kg & 39.6±1.84 cm and rohu Barisal was 1.20±0.17 kg & 40.1±1.85 cm. These four populations were collected from different locations *viz*. Khulna (22°49 0 N 89°33 0 E), Faridpur (23.50°N 89.83°E) and Barisal (22°48 0 N90°30 0 E) in Bangladesh and one was imported from India, known as Indian rohu. The distance from Khulna to Faridpur is 137.8 km, from Khulna to Barisal is 139.4 km and from Faridpur to Barisal is 128.4 km (www.google.com.bd/maps/ accessed on 16 July 2016). Modified CTAB method (Doyle, 1987) was used to isolate the total genomic DNA from pooled samples of rohu. Approximately 30 mg of caudal fin tissue was used to isolate the DNA. Caudal fin tissue was stored in 95% ethanol and cut into parts by sterilized scissors and initially washed in distilled water and then in ethanol. Nano Drop Spectrophotometer was used to determine DNA concentration. Fifteen primers were tested for RAPD amplification of which seven primers (Table 1) exhibited good banding patterns and variability.

PCR amplification

The PCR mix for 25 µl containing template DNA (25 ng) 2 µl, de-ionized distilled water 18.8 µl, Taq buffer A 10X (Tris with 15 mM MgCl2) 2.5 µl, primer (10 µM) 1.0 µl, dNTPs (2.5 mM) 0.5 µl, Taq DNA polymerase (5 U/µl) 0.2 µl. PCR amplification was done in an oil-free thermal cycler (Gene Atlas, Japan) for 46 cycles after initial denaturing at 94°C for 5 m, denaturing at 94°C for 1 m, annealing at 36°C for 30 s, extension at 72°C for 3 m and final extension at 72°C for 5 m.

Scoring and data analysis

The amplified products were separated electrophoretically on 1% agarose gel, observed on UV-transilluminator and photographed. The photographs were critically discussed on the basis of presence (1) or absence (0), size of bands and overall polymorphism of bands. Tables were drawn up for further investigation. RAPD analysis was then combined to create a single data matrix which was used for estimating linkage distance (D) among the populations based on Nei's method (1972) by using POPGENE 32 software (version 1.31) (Yeh et al., 1997). The dendrogram was created by UPGMA method (Unweighted Pair Group Method of Arithmetic Means) (Sneath and Sokal, 1973) using linkage distances by MEGA7 software (Kumar et al., 2016). Genetic distances were computed from frequencies of polymorphic markers for estimating genetic relationship among four populations of rohu.

RESULTS

RAPD polymorphism

These seven primers showed a total of 87 bands of which 35 were polymorphic with 48.38% polymorphisms among four geographically distant *L. rohita* populations. The size of the amplified DNA fragments ranged from 400 to 1250 bp (Fig. 1 & Table 1).

In addition to polymorphic bands, 8 unique bands were observed. The number, size, population and respective primer for each unique band were shown in Table 1 and Fig. 1. The unique bands were stable and specific for the respective population and thus could be used as a tool for characterization of a specific population. These results also indicate some degree of genetic diversity in four geographically distant populations of rohu.

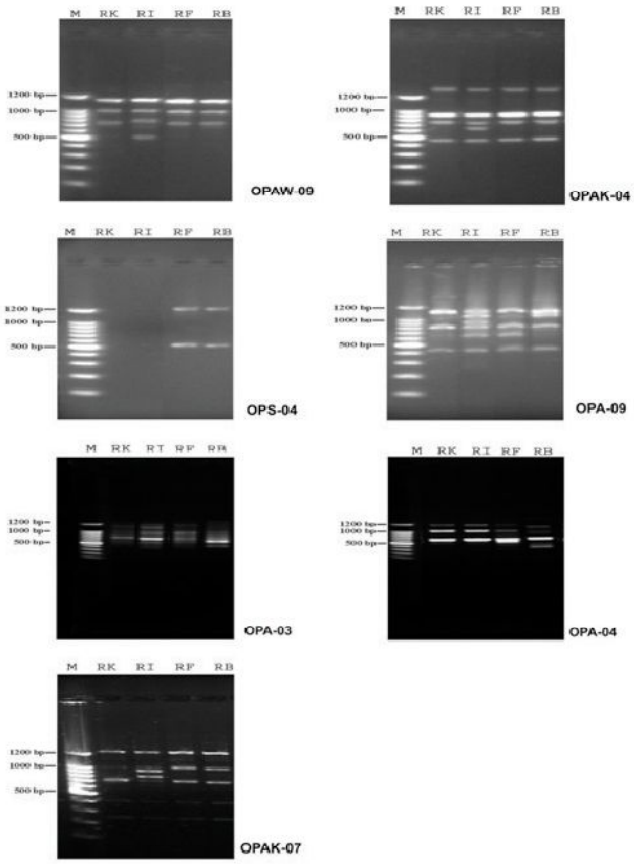

Fig 1. RAPD analysis with seven primers on four populations of *L. rohita* known as rohu Khulna= RK, rohu Indian=RI, rohu Faridpur=RF and rohu Barisal=RB. M= 1 kb DNA ladder

Genetic distances and genetic identity

The genetic distances ranged between 0.2595 and 0.7221 (Table 2). The highest genetic distance (0.7221) was found between rohu India and Barisal, while the lowest (0.2595) was among the three local populations of rohu Khulna, Faridpur and Barisal. The highest genetic identity (0.7714) was found in the three local populations of rohu Khulna, Faridpur and Barisal, and the lowest (0.4857) was between rohu India and rohu Barisal.

Table 2. Genetic identity and genetic distance among the four geographically different populations of rohu

Population ID	Rohu Khulna	Rohu India	Rohu Faridpur	Rohu Barisal
Rohu Khulna	****	0.7143	0.7714	0.7143
Rohu India	0.3365	****	0.6571	0.4857
Rohu Faridpur	0.2595	0.4199	****	0.7714
Rohu Barisal	0.3365	0.7221	0.2595	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

Table 1. Compilation of RAPD analysis in rohu from four geographically different locations

Primer codes	Sequences (5'—3')	Size ranges (bp)	Total bands	Number of polymorphic bands	Number of population-specific unique bands	Polymo-rphisms (%)	Average % polymorphism
OPAW-09	ACT GGG TCG G	500-1180	13	05	2 in rohu Indian	34.46	
OPAK-04	AGG GTC GGT C	490-1250	17	01	1 in rohu Indian	05.88	48.38
OPS-04	CAC CCC CTT G	500-1220	04	04	-	100.00	
OPA-09	GGG TAA CGC C	450-1170	16	04	1 in rohu Indian	25.00	
OPA-03	AGT CAG CCA C	500-1200	10	06	-	60.00	
OPA-04	AAT CGG GCT G	400-1190	12	08	1 in rohu Faridpur 1 in rohu Barisal	66.67	
OPAK-07	CTT GGG GGA C	700-1220	15	07	2 in rohu Indian	46.67	
Total			87	35	8		

Dendrogram (tree diagram)

Cluster analysis on the basis of DNA fingerprinting by RAPD was carried out by POPGENE 32 (version 1.31) among 4 geographically different populations of rohu. Dendrogram based on Nei's (1972) genetic distance was separated in three major clusters *viz.* C1, C2 and C3. Clusters C1 and C2 comprised the three local populations of rohu and on the other hand, rohu Indian populations created the new distant cluster - C3.

DISCUSSION

RAPD analysis

The four populations of rohu *viz*. Khulna, India, Faridpur and Barisal produced different banding pattern with seven primer combinations (Fig. 1). The average polymorphism was about 48.38%, revealing a moderate range of polymorphisms among these four populations of rohu.

Different average RAPD polymorphisms for rohu populations were reported by earlier studies such as 47.89% (Fayyaz et al., 2014), 45% (Barman et al., 2003), 27% (Khan et al., 2006) and 46.5% (Islam and Alam, 2004). In contrast, other Indian major carps showed a higher degree of polymorphism on the basis of RAPD markers *viz*. 85.82% (Basavaraju et al., 2014), 72.72% (Rasool et al., 2013) and 54.55% (Rahman et al., 2009). This may be due to non-conservative nature of genetic constituent that created variation in other carp except rohu.

The findings of the present study are more or less similar to the findings of previous researches. This study was conducted to estimate the genetic diversity of different rohu populations in Bangladesh in comparison with the Indian rohu population as well as within the three local rohu populations. Generally, genetic diversity among different populations is directly correlated with geographical distances (Rahman et al., 2009). Three artificially propagated rohu and rohu India had low genetic variability irrespective of geographical distribution.

Analysis of unique bands

The unique bands are found population-specific and could be used as markers for differentiation. A total of eight unique bands were observed of which six in the Indian rohu population and only one in rohu Faridpur and Barisal populations. This indicates that rohu India population is different from the other three, although significant phenotype differentiation was not found (Fig. 1 and Table 1).

Phylogenetic relationships among four rohu populations

Three rohu populations are locally cultured and they showed very little genetic distance in comparison to Indian rohu population. The geographical barriers lower the gene flow among the populations and thus genetic distances increase remarkably (Wright, 1943). There is no distinct geographical barrier among the three rohu populations and perhaps the brood fishes of that cultured rohu came from the same population or same tributary and they showed lower genetic variability among themselves. Indian rohu showed differences due to different geographical area, habitat environment and ecological parameters in India (Fig. 2).

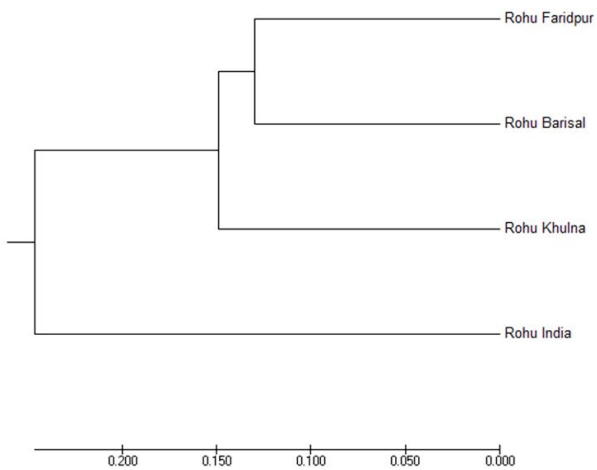

Fig 2. The dendrogram was inferred using the UPGMA method (Sneath and Sokal, 1973). The optimal tree with the sum of branch length = 0.77158333 is shown. Dendrogram was conducted in MEGA7 (Kumar et al., 2016)

The present study and the findings of the previous ones showed that the genome of rohu is conservative, and moderate variability was found in rohu. The polymorphism of rohu below 60% indicates a low amount of genetic variability among different populations. Therefore, for an improved breeding program, distant populations are needed to avoid the inbreeding depression of rohu.

CONCLUSIONS

This research indicated that artificially propagated rohu showed a low amount of genetic diversity irrespective of geographical distribution. So for improved breeding program, these artificially propagated rohu would be a wrong choice. Natural diversified populations would be a better choice to improve the breeding program.

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Sažetak

GENETSKA RAZLIČITOST POPULACIJA INDIJSKOG ŠARANA (*Labeo rohita*) ODREĐENA POMOĆU RAPD MARKERA

Genetička raznolikost među četiri geografski udaljene populacije indijskog šarana (*Labeo rohita*) - rohu Khulna, rohu India, rohu Faridpur i rohu Barisal - određena je pomoću RAPD markera. Uzorak na gelu je pokazao da je populacija rohu India genetski različita od triju ostalih. Ukupno je pronađeno 87 crtica u četiri rohu populacije pri čemu je 35 crtica bilo polimorfno, što ukazuje na 48,38% polimorfizma s prosjekom od 12 crtica po početnici. Dužina amplificiranih fragmenata DNK kretala se u rasponu od 400 do 1250 bp. Osam jedinstvenih crtica je uočeno u četiri populacije od kojih je šest pronađeno u rohu India populaciji. Genetska udaljenost je bila najviša (0,7221) i genetska identičnost je bila najniža (0,4857) između populacija rohu India i rohu Barisal. Među tri lokalne populacije najniža genetska udaljenost i najviša genetska identičnost utvrđena je između populacija rohu Khulna i rohu Faridpur. UPGMA filogenetsko stablo odijelilo je četiri populacije indijskog šarana u tri glavna odjeljka - C1, C2 i C3. Rohu Indija nalazi se na potpuno drugačijem odjeljku C3, rohu Khulna je smještena u C2, a ostale dvije populacije u C1 odjeljak.

Ključne riječi: RAPD, genetska različitost, *Labeo rohita*

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