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GENETIC DIVERSITY OF OLIVE BARB (*Systomus sarana,* Hamilton, 1822) FROM DIFFERENT LOCATIONS OF BANGLADESH

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ABSTRACT

Olive barb (*Systomus sarana*) was collected from three different locations viz. Kuliarchar, Bhairab and Bikrampur in Bangladesh to elucidate genetic diversity by random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR). Six arbitrary oligonucleotide RAPD primers were used to amplify the DNA from each population. A total of 52 bands were produced in 3 olive barb populations where 28 bands were polymorphic indicating 53.84% polymorphisms in those three populations with an average of 9 bands per primer. The molecular size of the amplified DNA fragments ranged between 300 and 2300 bp. 12 unique RAPD bands were observed in the three populations. The bands are specific and stable and thus could be used to characterize each germplasm. The values of pair-wise genetic distances ranged between 0.5232 and 0.8109 with some degrees of genetic variation among the populations. The highest genetic distance (0.8109) was observed between olive barb collected from Kuliarchar and Bikrampur, while the lowest (0.5232) was found between olive barb collected from Kuliarchar and Bhairab. The UPGMA dendrogram segregated three samples of olive barb into two major clusters C1 and C2. The Kuliarchar-Bhairab population pair of olive barb was genetically closer than that of Kuliarchar-Bikrampur. Therefore, RAPD analysis can be used to identify the genetic diversity in olive barb. This information may be used for improved breeding programme and conservation of native olive barb.

How to Cite

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INTRODUCTION

Olive barb (*Systomus sarana*) has high nutritional value and market demand in Asian countries, especially in Bangladesh (Chakraborty et al., 2006).The digestibility and biological value of flesh of this species is very high. Olive barb is widely distributed through the Indian sub-continent including Bangladesh, India, Pakistan, Nepal, Bhutan, Sri-Lanka, Afghanistan and Thailand (Talwar and Jhingran, 1991; Bhat, 2004; Chakraborty et al., 2006; Froese and Pauly, 2007; Jena et al., 2007). This small indigenous species (SIS) was found abundantly in rivers, streams, ponds, beels, ditches and floodplains in the South Asian countries (Bhat, 2004; Chakraborty et al., 2006; Jena et al., 2007). Olive barb can be used as ornamental fish because of its silver-colored body and hardy nature. This species can also be used as biological control in aquaculture because of its herbivorous nature. These can eradicate aquatic weeds, especially lemna species from ponds (Kashyap et al., 2014).

The present status of this fish has been declared as critically endangered (IUCN Bangladesh, 1998; Ameenet al., 2000; Hussain and Mazid, 2004). In addition, Mukherjee et al. (2002) categorized the species as a vulnerable species. Nowadays, this species has been seriously declined or on the verge of extinction due to overexploitation and various ecological changes in its natural habitats which severely af-

fects biodiversity (Mukherjee et al., 2002; Chakraborty et al., 2006). As a consequence, genetic variability is becoming narrow day by day. For improved breeding, genetic variability is a prime prerequisite. Therefore, there is an urgent need to elucidate the genetic variability of our native olive barb.
The random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique allows detection of polymorphisms by randomly amplifying independent multiple regions of the genome through PCR using single arbitrary primers (Williams et al., 1990 and Welsh and McClelland, 1990). RAPD-PCR technique is simple, fast and sensitive. This technique allows identifying genetic variation without prior knowledge of DNA sequences. RAPD-PCR has been widely employed in fisheries studies (Akter et al., 2010). Genetic diversity enables a species or a stock to adapt to changing their environment. Genetic data are needed to understand the structure of stocks so that appropriate decisions can be made towards conservation of the species (Kimberling et al., 1996). RAPD markers have been used 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 olive barb population collected from geographically distant natural habitats by RAPD-PCR analysis.

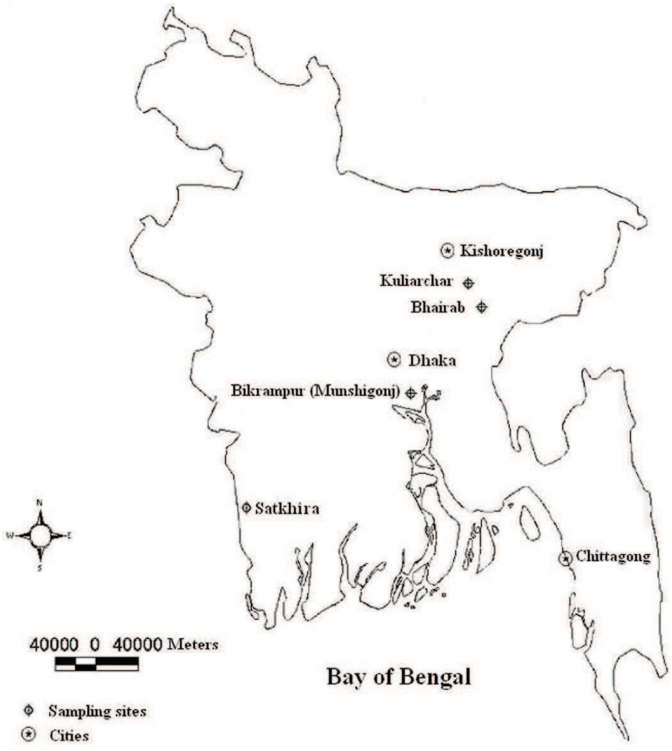

Fig 1. Sampling points of olive barb from three geographically distant locations in Bangladesh

MATERIALS AND METHODS

Sample collection

Olive barb was collected from three geographically distant locations *viz*. Kuliarchar (24.1542°N; 90.9000°E), Bhairab (24.0500°N; 90.9875°E) and Bikrampur, also known as Munshigong (23.4583°N; 90.5417°E) in Bangladesh in the month of June 2014 (Fig. 1). Random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique was used to identify the genetic diversity among them. The distance from Kuliarchar to Bhairab is 15.8 km, Kuliarchar to Bikrampur is 123 km and Bhairab to Bikrampur is 107.3 km (www.google.com.bd/maps/ accessed on 16 November 2014). Kuliarchar, Bhairab and Bikrampur are 94.4 km, 80.7 km and 37.9 km away from the capital city, Dhaka, respectably.

Collection of sample &isolation of genomic DNA

Total genomic DNA was isolated from pooled samples of olive barb by using a modified CTAB method (Doyle and Doyle, 1987). About 30 mg of caudal fin tissue was stored in 95% ethanol then cut into small pieces with the help of sterilized scissors and washed well initially in distilled water and then ethanol. These were dried on fresh tissue paper to remove any other foreign DNA.

Quantification of isolated DNA

Quantification was done by comparing DNA with the standard DNA on 1% agarose gel electrophoresis and by estimating the absorbance of DNA by spectrophotometer (Analylikjena, Specord 50, Germany). The A260/280 readings for DNA samples were 1.5–1.8.

Primer selection

Primarily, 20primers were tested for RAPD amplification of which six primers (Operon Technologies, USA and Sigma USA) named OPA-1 (CAG GCC CTT C), OPA-2 (TGC CGA GCT C), OPA-7 (GAA ACG GGT G), OPA-8 (GTG ACG TAG G) Primer 24 (GGT CGG AGA A) and Primer 25 (GTG CGG AGA A) exhibited good quality banding patterns and sufficient variability. These primers were selected for further analysis.

PCR amplification

The PCR reaction mix for 25 µl contained template DNA (25 ng) 2 µl, de-ionized distilled water 18.8 µl, Taq buffer A 10X (10 mM Tris with 15 mM MgCl2) 2.5 µl, primer (10 µM) 1.0 µl, dNTPs (10mM) 0.5 µl, Taq DNA polymerase (5 U/µl) 0.2 µl. PCR amplification was done in an oil-free thermal cycler (Biometra UNOII, Germany) for 46 cycles after initial dena-

turing at 94°C for 5 m, denaturing at 94°C for 1m, annealing at 36°C for 30 s, extension at 72°C for 3 m and final extension at 72°C for 5 m.

Gel electrophoresis, scoring and data analysis

Amplified DNA was run through 1% agarose gel electrophoresis for the observation of quantity of DNA. All the samples showed adequate quantity of DNA produced by PCR amplification. DNA ladders (1 kb) were electrophoresed alongside the RAPD reactions as marker. DNA bands were observed on UV-trans illuminator and photographed by a gel documentation system. The photographs were critically analyzed on the basis of presence (score 1) or absence (score 0) of band, size of bands and overall polymorphism of the bands. The values of pair-wise genetic distances were analyzed by using computer software "POPGENE32" (version 1.31) among 3 geographically different populations of olive barb from data of six RAPD primers.

RESULTS

Initially 20 decamer primers were tested of which six primers *viz*. OPA-1, OPA-2, OPA-7, OPA-8, primer 24 and primer 25 exhibited good quality banding patterns and sufficient variability with less smearing. These primers were selected for further RAPD analysis of three geographically distant populations of olive barb (Table 1). These six primers produced a total of 52 bands of which28 were polymorphic with 53.84% polymorphisms among three geographically distant olive barb populations. The size of the amplified DNA fragments was ranging from 300 to 2300 bp (Fig. 2).

In addition to polymorphic bands, 12 unique bands were observed. The number, size, population and respective primer for each unique band were shown in Table 1 and Figure 2. 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 degrees of genetic diversity in three geographically distant populations of olive barb.

Genetic distances

The values of pair-wise genetic distances were analyzed by using computer software "POPGENE32" (version 1.31) using total RAPD fragments. The genetic distances ranged between0.5232and0.8109 (Table 2). The highest genetic distance (0.8109) was found between Kuliarchar and Bikrampur populations, while the lowest (0.5232) between Kuliarchar and Bhairab populations.

Genetic identity

In the present study, interpopulation genetic identity was analyzed by computer software "POPGENE 32" (version 1.31).It was found between 0.4444 and 0.5926 (Table 2). The highest genetic identity (0.5926) was found in Kuliarchar and Bhairab populations, while the lowest (0.4444) in Kuliarchar and Bikrampur populations. Shannon's information index was determined as 0.4715±0.2843, Nei's (1972) gene diversity as 0.3292±0.1985 and effective number of alleles as 1.5926±0.3573 in the interpopulation of olive barb using computer software "POPGENE 32" (version 1.31) (Table 3).

Table 1. Compilation of RAPD analysis in olive barb from three geographically different locations in Bangladesh

Primer codes	Sequences (5'—3')	Size ranges (bp)	Total bands	Number of Polymorphic bands	Number and size (bp) of population specific unique bands	Polymorphisms (%)	Average % Polymorphism
OPA-1	CAG GCC CTT C	350-2100	13	01	1 (2100) in BK	7.69	53.84
OPA-2	TGC CGA GCT C	650-1500	06	06	2 (850, 1500) in BK	100	
OPA-7	GAA ACG GGT G	400-1250	07	07	1 (850) in KL 2 (500, 750) in BH 2 (650,1150) in BK	100	
OPA-8	GTG ACG TAG G	750-2300	09	06	-	66.66	
Primer 24	GGT CGG AGA A	300-1400	09	06	2 (300, 420) in BH	66.66	
Primer 25	GTG CGG AGA A	350-1350	08	02	2 (600, 900) in BH	25	
Grand total			52	28	12		

KL=11, BH=22, BK=19

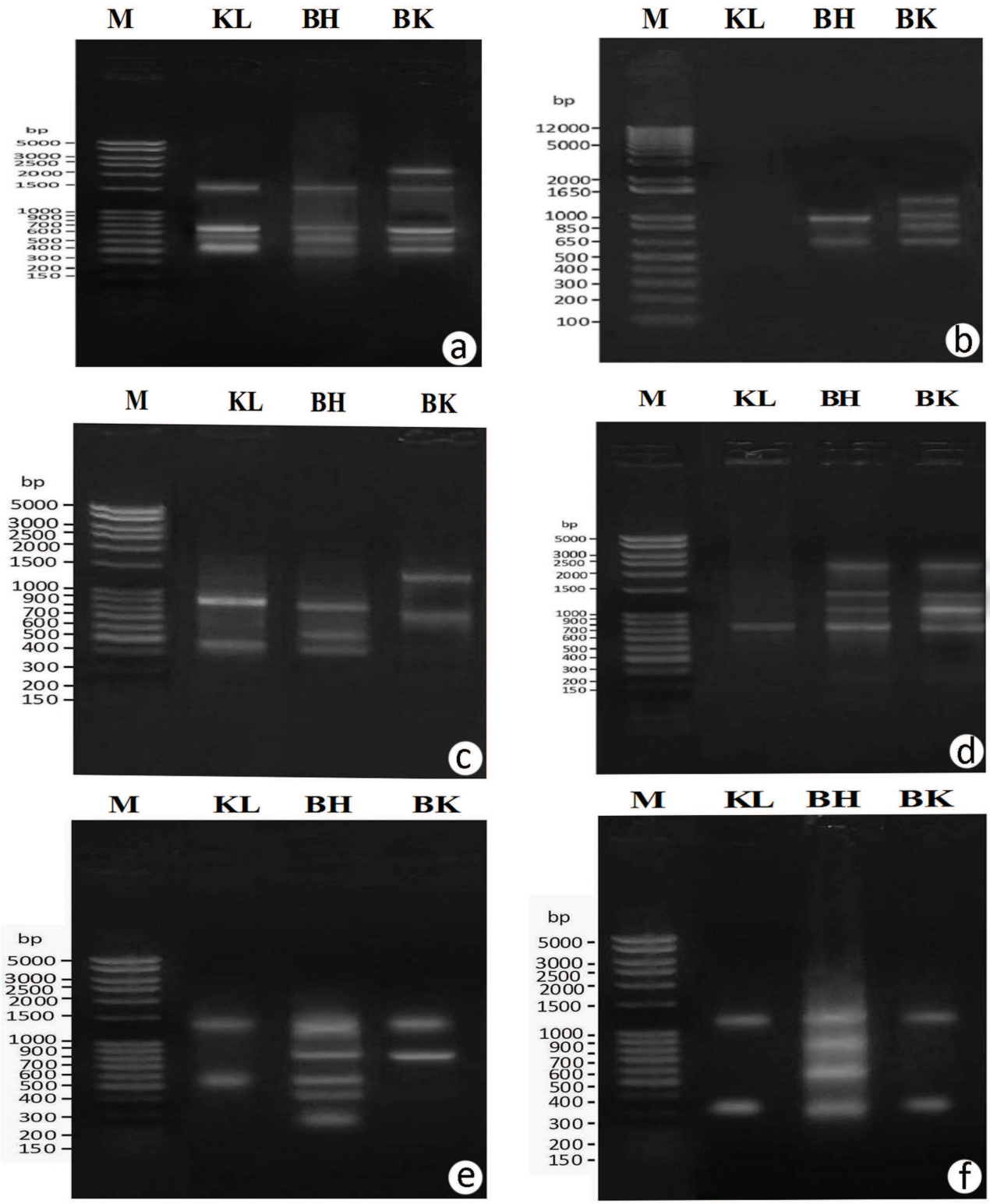

Fig 2. (a-f). RAPD analysis with six primers on three populations of olive barb from different location in Bangladesh. a. Primer OPA-1, b. Primer OPA-2, c. Primer OPA-7, d. Primer OPA-8, e. Primer 24 and f. Primer 25. M= 1 kb DNA ladder, KL=Olive barb from Kuliarchar, BH= Olive barb from Bhairab and BK= Olive barb from Bikrampur

Table 2. Genetic distances and genetic identity of olive barb sampled from three geographically distant locations in Bangladesh

Population ID	Olive barb from Kuliarchar	Olive barb from Bhairab	Olive barb from Bikrampur
Olive barb from Kuliarchar	****	0.5926	0.4444
Olive barb from Bhairab	0.5232	****	0.4815
Olive barb from Bikrampur	0.8109	0.7309	****

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

Table 3. Interpopulation genetic analysis of olive barb indicates the number of allele observed, effective number of allele, gene diversity and Shannon's information index of these populations

Interpopulation Genetic analysis	Observed number of alleles (na)	Effective number of alleles (ne)	Nei's (1973) gene diversity (h)	Shannon's Information index (i)
Mean	1.7407	1.5926	0.3292	0.4715
SD	0.4466	0.3573	0.1985	0.2843

Cluster analysis (Tree diagram)

Cluster analysis on the basis of DNA fingerprinting by RAPD was carried out. Dendrogram based on Nei's (1972) genetic distance was constructed using Unweighted Pair Group Method of Arithmetic Means (UPGMA) segregating three geographically distant populations of olive barb into two major clusters *viz.* C_1 and C_2. Olive barb from Kuliarchar and Bhairab formed cluster 1 (C_1), while those from Bikrampur formed cluster 2 (C_2)(Fig.3).

DISCUSSION

RAPD analysis

The three populations showed different RAPD banding pattern with six prime combinations (Fig. 2). The average polymorphismwas about 53.84% indicating moderate range of polymorphisms among three populations of olive barb.
Akter et al. (2010) reported a high level of polymorphisms (89.58 %) in four barb individuals of which three were *S.*

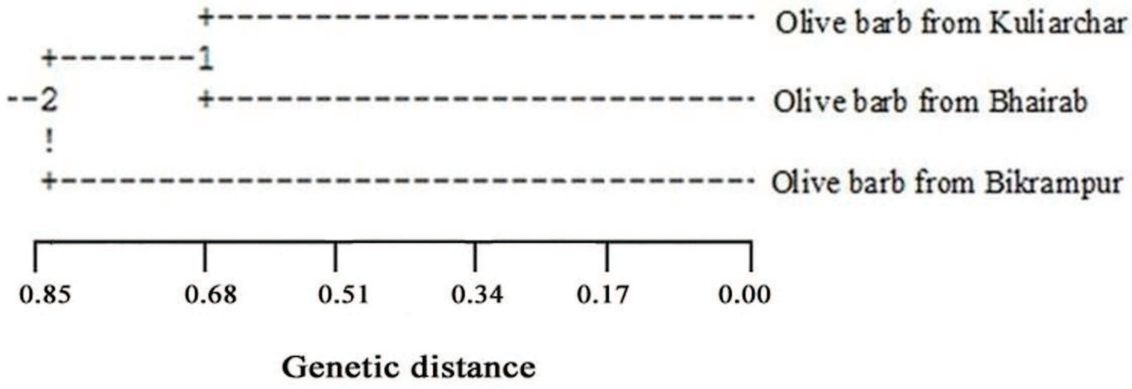

Fig 3. UPGMA dendrogram based on RAPD analysis of three populations of olive barb from different locations in Bangladesh

sarana populations (local barb) collected from the Chalan Beel, Mogra River and private hatchery- Brahmaputra Fish Seed & Hatchery Complex, Mymensingh, Bangladesh. Another one was *Barbonymus gonionotus* (Thai silver barb) collected from the same hatchery. Thai silver barb is phenotypically quite different from local barb and, therefore, polymorphisms must be higher in comparison to local olive barb. However, they did not report the percent of polymorphism among three local barbs alone. The percentage of polymorphism among three local barbs will likely be decreased from 89.58%.

Polymorphisms based on RAPD analysis were reported in other fishes earlier. Mostafa et al. (2009) reported 57.69% polymorphisms among two riverine and one hatchery stock of *Labeo calbasu* collected from Padma, Jamuna and hatchery. Barman et al. (2003) indicated 45% polymorphisms in four different populations of *Labeo rohita*, *Catla catla*, *Labeo calbasu* and *Cirrhinus mrigala*. Islam and Alam (2005) found 46.5% of polymorphisms in four different populations of *L. rohita*. The polymorphisms are ranging between 45 to 60%. In the present study, 53.84% polymorphism was found (Table 1). Therefore, the average polymorphisms of order Cypriniformes is ranging between 45 to 60%.

Besides polymorphic bands, 12 unique bands were found. The term unique band means that a band found with a specific primer in an individual is absent in other individuals with the same primer. Unique bands are very stable and specific to each germplasm. These unique bands could be used as a marker for respective germplasm. This information regarding RAPD unique bands may be used to patent native barbs.

Phylogenetic relationships among three olive barb populations

The dendrogram shows that the genetic distance between Bhairab and Kuliarchar populations is about 0.6. In contrast, it is 0.85 between those of Bhairab and Kuliarchar populations and Bikrampur population (Fig 3). Bhairab and Kuliarchar places are geographically very close at about 15 km distance, while Bikrampur is about 70 km from these places, isolated geographically by two rivers from the other two populations. The geographical barriers lower the gene flow among the populations and thus the genetic distances increase remarkably. On the other hand, Bhairab and Kuliarchar have little geographical barrier and gene flow is frequent thus having less genetic distance.

Geographical distance is an important factor influencing the genetic relatedness of populations (Wright, 1943). Bhat et al. (2014) reported that genetic diversity is primarily dependent on geographical isolation and there is a significant correlation between genetic diversity and geographical distance. Therefore, the Bikrampur population is more diverse due to geographical distance. This information will be helpful to select competent parents for improved breeding programme and conservation of the gene stock of native olive barbs.

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Sažetak

GENETSKA RAZLIČITOST AZIJSKOG MASLINASTOG CIPRINIDA (*Systomus sarana*, Hamilton, 1822) IZ NEKOLIKO RAZLIČITIH LOKACIJA U BANGLADEŠU

Analizirani su uzorci Azijskog maslinastog ciprinida (*Systomus sarana*) s tri različite lokacije Kuliarchar, Bhairab i Bikrampur u Bangladešu radi utvrđivanja genetske raznolikosti putem slučajno amplificirane DNA pomoću lančane reakcije polimeraze (PCR-RAPD). Šest proizvoljnih oligonukleotida RAPD početnica korišteno je za amplifikaciju DNA iz svake populacije. Ukupno 52 benda su producirana na 3 populacije *S. sarana*, od čega je 28 bendova bilo polimorfno, što ukazuje na 53,84% polimorfizma u te tri populacije, s prosjekom od 9 bendova po početnici. Veličina amplificiranih fragmenta DNA kretala se u rasponu između 300 i 2300 bp. Uočeno je 12 jedinstvenih RAPD bendova u tri populacije. Bendovi su specifični i stabilni te stoga mogu biti korišteni za karakterizaciju svake germplazme. Vrijednosti genetskih udaljenosti bila je u rasponu između 0,5232 i 0,8109 s određenim stupnjem genetske varijacije između populacija. Najveća genetska udaljenost (0,8109) zabilježena je između *S. sarana* prikupljenih iz provincija Kuliarchar i Bikrampur, dok je najniža genetska udaljenost (0,5232) pronađena između jedinki prikupljenih iz provincija Kuliarchar i Bhairab. UPGMA dendrogram razdvaja tri uzoraka *S. sarana* u dva glavna klastera C1 i C2. Populacije Kuliarchar-Bhairab su genetski bliže nego Kuliarchar-Bikrampur. Stoga se RAPD analiza može koristiti za identifikaciju genetske raznolikosti vrste *S. sarana*. Ovi podaci se također mogu koristiti za poboljšanje uzgojnog programa i očuvanje populacija u otvorenim vodama.

Ključne riječi: tehnika RAPD-PCR, genomska DNA, DNA identifikacija, genetska raznolikost

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