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# Assessment of Genetic Variation among Hatchery Populations of the Indian Major Carp, *Catla*, *Labeo catla* (Hamilton, 1822) Using RAPD Markers

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

Genetic variation is regarded as a key factor for adaptation to the changing environment, therefore, in this study, genetic variations of *Labeo catla* were analysed using Randomly Amplified Polymorphic DNA (RAPD). *The Catla* (*Labeo catla*) seeds were procured from three distinct

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geographical locations *viz*., West Bengal, Andhra Pradesh, and Karnataka. The growth performance of Catla were conducted for period of 120 days under different environmental and management conditions. Growth trials resulted better performance of Andhra Pradesh stock followed by West Bengal and Karnataka. For assessment of genetic variation 140 decamer primers were screened, out of which, 6 primers (OPA02, OPA04, OPA07, OPA10, OPB07 and OPB10) that yielded consistent and significant polymorphism were selected for RAPD analysis which indicated West Bengal stock being more polymorphic (60.55%) in comparison with Karnataka and Andhra Pradesh stocks. Higher Shannon's diversity (0.2957) and Nei's heterozygosity (0.2017) was observed in the West Bengal stock. In terms of genetic identity and genetic distance, Karnataka and Andhra Pradesh stocks showed a genetic distance of 0.7101 and a minimum Genetic identity of 0.4916, while West Bengal and Karnataka stocks indicated a maximum genetic identity of 0.6290 and a minimum genetic distance of 0.4637. The study also showed Karnataka stock being closely related to West Bengal stock when compared to Andhra Pradesh stock. The $r^2$ values of 0.00238 showed a positive correlation between the percentage of polymorphism and mean body weight attained by different stocks of *Labeo catla*.

*Keywords: Labeo catla; laboratory rearing; polymorphism; genetic variation; RAPD marker.*

## 1. INTRODUCTION

Fisheries and Aquaculture remains to be an important source of food, nutrition, income, and livelihood to about 28 million fishers and fish farmers at the primary level and almost twice the number along the value chain. India is the third largest fish producing country and the second largest aquaculture fish producer in the world. India contributes about 8.92 % to the global fish production (Handbook of Fisheries Statistics, 2023). Indian major carps are the most cultivable fish species in India contributing to about 87% of the total freshwater aquaculture production of the country (Ayyappan & Jena, 2003). Around 70% of Indian fish production comes from inland waters, of which, nearly 65% comes from aquaculture (IBEF, 2022). Catla being an Indian major carp and one of the major aquaculture species of India, Bangladesh and Myanmar (Burma), is considered as the second most important species after *Labeo rohita* (Rohu) and is observed to be the surface feeder component in Indian major carp polyculture systems that involve six-species composite culture. The Catla was formerly listed as the only species in the genus *Catla* with the synonym *Gibelion*. Lately, the catalogue of fishes moved the species to the new genus *Labeo* (Stout et al*.,* 2016). Molecular techniques based on DNA sequence polymorphism are now used in studies related to population genetics, systematic and molecular taxonomy to seek answers to systematic related problems which play an important role in understanding the basis of polymorphism of a species, species diagnostics and population differentiation (Avise, 1996). Undoubtedly, genetic variability in fishes has been reported to be highly valuable in aquaculture and fisheries management, identification of stocks, stock enhancement, selection of breeding programmes, ecology restoration, estimation of genetic contributions in stocks, and management measures for sustainable yield and preservation of genetic diversity (Danish et al., 2012; Dinesh et al., 1993; Tassanakajon et al., 1997; Tassanakajon et al., 1998). The application of genetic markers has allowed rapid progress in aquaculture investigations of parentage assignments; genetic variability and inbreeding; species and strain identification; and the construction of high-resolution linkage maps for aquaculture species (Liu and Cordes, 2004). Random Amplified Polymorphic DNA (RAPD) has the advantage that no prior knowledge of the genome is necessary for successful application. The RAPD markers method is an efficient tool for differentiating geographically and genetically isolated populations. It has been employed to verify the existence of populations of species that have arisen either through genetic selection under different environmental conditions or as a result of genetic drift (Fuchs et al., 1998). RAPDs are particularly useful for studying the genetic structure of populations as they reveal polymorphisms in non-coding regions of the genome. Random primers produce RAPD bands that have been used extensively as molecular markers (Vucetich et al., 2001).

The current study employs the RAPD technique to understand the variation of nuclear DNA in the three stocks of Catla (*Labeo catla*) seeds procured from different geographical locations with the following objectives: to conduct growth trials; to screen and select primers to reveal

better polymorphism; and to study the genetic diversity in different stocks of Catla (*Labeo catla*).

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

Catla (*Labeo catla*) procured from different geographical locations *viz*., Kolkata (West Bengal), Mysore (Karnataka) and Kaikaluru (Andhra Pradesh), which were genetically and reproductively isolated for all practical purposes, were maintained by using the laboratory and field facilities available at Fisheries Research and Information Centre (Inland) in Hebbal, Bangalore (Fig. 1). It was observed that the stock in these centres has been maintained for more than 4-5 or more generations, and is almost genetically and reproductively isolated.

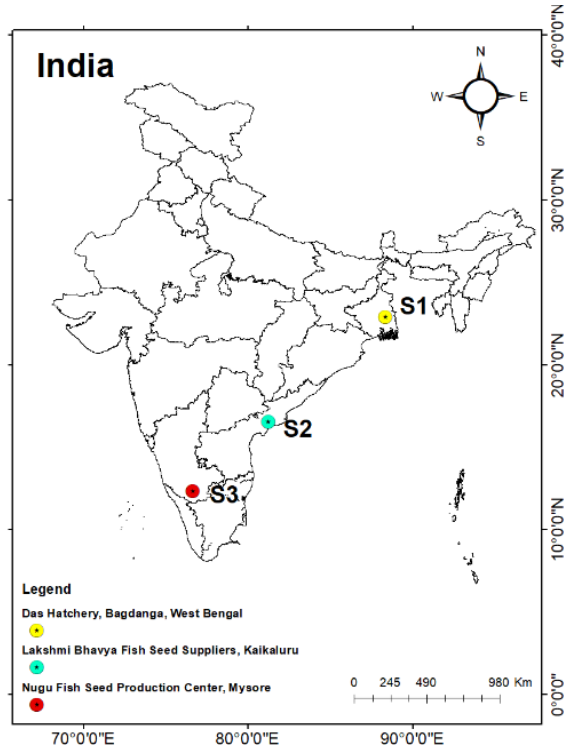



**Fig. 1. The Map Shows different stocks of Catla (*Labeo catla*) evaluated with their source**

### 2.2 Laboratory Rearing Period of *Labeo catla*

Rearing of Catla (*Labeo catla*) stocks was conducted in three separate 1000 litre capacity FRP tanks for a period of 120 days with a stocking density of 100nos./tank. The initial average length and weight of fish were recorded as 2.01 to 2.56±0.5 cm and 1.52 to 1.55±1.0 g respectively. The fishes were fed at 5% body weight with a control feed. During the experiment the water quality parameters such as Temperature, Dissolved Oxygen, pH, Electrical Conductivity, Ammonia, Nitrate and Nitrite were estimated (APHA et al., 2017). During the experimental period, water temperature and pH were recorded daily and were found to be within normal range; other physic-chemical parameters such as dissolved oxygen, electrical conductivity, ammonia, nitrite, and nitrate were analysed fortnightly. Excess feed and faecal matter were siphoned out of the FRP tanks once in two days, and 50% of water was replenished. The length and weight of fishes were recorded every 15 days. At the end of 120 days of rearing period, the final length and weight of all the surviving fishes from different stocks were recorded.

### 2.3 Molecular Analysis Using RAPD Markers

**Molecular data:** The fin tissue samples of were collected from all the stocks at the end of 120 days of laboratory rearing period for genetic studies. A small portion of the pectoral and pelvic fins was cut using sterile scissors and preserved in 95% ethanol prior to the preservation of fish samples in 10% formalin. The ethanol-preserved muscle samples were stored under refrigerated conditions till the extraction of genomic DNA.

**Extraction of DNA and RAPD- PCR amplification:** Total Genomic DNA was isolated from the pectoral and pelvic fin clips using the Phenol-Chloroform-Isoamyl-Alcohol (PCIA) extraction protocol (Sambrook et al., 1989). A total of 140 decamer primers obtained from Eurofins Genomics, Bangalore, India (OPA 1-20, OPB 1-20, OPC 1-20, OPF 1-20, OPG 1-20, OPP 1-20 and OPE 1-20, with ~ GC content of 70%, were used for screening different stocks. The screening was carried out using an optimized RAPD-PCR protocol for a minimum of five samples from each stock for each primer. Six primers that yielded the maximum number of consistent reproducible bands were selected for PCR analysis (Table 1), and were repeated a minimum of five times to confirm the amplification results. Polymerase Chain Reaction (PCR) amplification conditions were performed on each DNA sample in a total reaction volume of 20 µl containing 25 ng of genomic DNA, 2 µl of 10 × Assay buffer (containing 25mM $MgCl_2$), 200 µM dNTPs, 1U of Taq DNA polymerase, and 10 pM of random decamer primers (Eurofins Genomics

**Table 1. Sequence of oligonucleotide primers selected for the study**

| Sl. No. | Locus Name | Primer sequence (5' to 3') | Nucleotide Length | GC content (%) | Annealing $T_A$ [˚C] |
|---|---|---|---|---|---|
| 1. | OPA-02 | TGCCGAGCTG | 10 | 70.00 | 33 |
| 2. | OPA-04 | AATCGGGCTG | 10 | 60.00 | 28.9 |
| 3. | OPA-07 | GAAACGGGTG | 10 | 60.00 | 28.9 |
| 4. | OPA-10 | GTGATCGCAG | 10 | 60.00 | 28.9 |
| 5. | OPB-07 | GGTGACGCAG | 10 | 70.00 | 33 |
| 6. | OPB-10 | CTGCTGGGAC | 10 | 70.00 | 33 |

Bangalore, India) with the following profile: initial denaturation at 95 °C for 5 min, followed by 45 cycles each consisting of denaturation at 94 °C for 1 min, primer annealing at 36 °C for 1 min, and primer extension at 72 °C for 2 min, and with a final extension at 72 °C for 10 min in a Master cycler gradient (Eppendorf, USA) PCR machine. RAPD-PCR was conducted using the selected best six primers for 30 samples of catla (*Labeo catla*) from each stock. A negative control consisting of all the reaction components except template DNA was also included for each amplification. Electrophoresis for all samples was performed in 1.5 % agarose gel (added with ethidium bromide) in a casting tray and gel picture was documented in a Gel Doc System (BIO-RAD) for future reference.

## 2.4 Statistical Data Analysis for RAPD

The RAPD fingerprint generated for all samples was compared within and between populations. The genotypes were determined by recording the presence (1) or absence (0) of the bands and neglecting the weak and unresolved bands. Nei's (1978) unbiased genetic identity (I) and genetic distance (D) values between catla (*Labeo catla*) populations were calculated using the data generated from RAPD profiles using POPGENE 1.32 (Yeh et al., 1999). Genetic distance values were utilized to construct a dendrogram through clustering analysis (UPGMA) to determine the relationship between catla (*Labeo catla*) populations. For more accuracy, band scoring was performed by two independent persons. Bands not identified by the two readers were considered to be non-scorable. The scores obtained using all selected primers in the RAPD analysis were then pooled for constructing a single data matrix. This was used for estimating the proportion of polymorphic loci and genetic distance, and the construction of a dendrogram (Nei, 1978).

The percentage of polymorphism was calculated for each stock for each selected primer using the formula:

$$\text{Percentage of polymorphism} = \frac{\text{Total number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

Comparisons were carried out between samples amplified by the same primer in a pairwise manner. Heterozygosity was calculated by Nei (1973), and unbiased measures of genetic identity and genetic distance were calculated by Nei (1978). The UPGMA dendrogram of the population was constructed based on Nei (1972). The Nei's (1973) genetic diversity analysis was undertaken to determine the genetic structure of the populations. The $G_{st}$ statistics were used to measure the genetic differentiation between subpopulations relative to the genetic diversity in the total population. Genetic differentiation ($G_{st}$) was calculated by using the following formula:

$$\text{Genetic differentiation } (G_{st}) = 1 - \frac{Hs}{Ht}$$

Where; Hs is sample gene diversity; Ht is total gene diversity.

The level of gene flow (Nm) was measured following Nei's (1973) gene diversity statistics. Gene flow was indirectly estimated among the populations by using the formula:

$$\text{Estimation of gene flow (Nm)} = \frac{0.5\ (Gst)}{Gst}$$

(McDermott and McDonald, 1993)

Shannon's diversity index was calculated to provide a relative estimate of the degree of genetic variation within each population using POPGENE 1.32 Software (Yeh et al., 1999). One-way ANOVA was used to analyse data from the growth trials and water quality parameters. Percentage polymorphism and the growth of different stocks were correlated using $r^2$ (Correlation coefficient of determination) to deduce the correlation between the percentage

of polymorphism and the mean average body weight attained by different stocks of catla using MS Excel.

$$r = \frac{n(\Sigma xy) - (\Sigma x)(\Sigma y)}{\sqrt{[n\Sigma x^2 - (\Sigma x)^2][n\Sigma y^2 - (\Sigma y)^2]}}$$

Where; r - Sample Correlation Coefficient, n - Sample size, x - Value of the independent variable (Percentage of polymorphism), y - Value of the dependent variable (Average growth rate of different stocks of fish).

## 2.5 Genetic Distance/Identity

Unbiased measures of genetic identity and genetic distance were computed following the protocol of Nei (1972) and Nei (1978). The RAPD band patterns of individuals were compared within and between populations; and between different stocks. Pair-wise similarities ($S_{AB}$) between individual fish were calculated using the data from polymorphic primers. $S_{AB}$ for shared DNA fragments between two individuals A and B was calculated as follows (Lynch, 1991):

$$S_{AB} = 2\ N_{AB} / (N_A + N_B)$$

Where; $N_{AB}$ is the number of DNA fragments in common between individuals A and B; $N_A$ and $N_B$ are the total number of fragments possessed by individuals A and B; One DNA fragment or RAPD marker was interpreted to represent one locus.

## 3. RESULTS

The results of growth trials indicated that West Bengal stock had the highest survival rate in all three FRP tanks (85%), followed by Karnataka stock (79%) and Andhra Pradesh stock (74%). In the laboratory rearing or fish growth trials, the trends in growth rate are shown in Table 2 and Fig. 2&3. Six primers *viz*., OPA 02, OPA 04, OPA 07, OPA 10, OPB 07 and OPB 10 resulted in amplification of more than five bands and were subsequently selected for the analysis. The selected decamer primers exhibited good technical resolution with quality banding patterns that were clear, consistent, easy to score, and hence were used for estimating the genetic diversity.

## 3.1 Water Quality Parameters

All water quality parameters were in the optimum range during the laboratory rearing period. The Temperature range was 21-26 °C, Dissolved Oxygen range 5.9-8.9 ppm, pH ranges 6.7-8.5, Electrical Conductivity range 0.11-0.98 S/m and Ammonia, Nitrate, and Nitrite concentrations ranging 0.05-0.77, 0.01-0.05, and 0.06-0.30 ppm respectively (Table 3).

## 3.2 RAPD profile of Different Stocks of *Labeo catla*

Among the selected six primers OPA-02, OPA-07, OPA-10, and OPB-10 have given a maximum number of 5 bands while OPB-07 has given a minimum number of 3 bands. Selected primers revealed better polymorphism in the different stocks. Of the six best primers chosen, OPA-10 has given a maximum polymorphism of 80 percent whereas OPB-07 has given a minimum polymorphism of 22 percent between different stocks. West Bengal stock, OPA-02 and OPA-10

**Table 2. Mean Length and Weight attained by different stocks of *Catla* during the study period**

| Sl. No. | Stocks Name | Initial length | Initial weight | Final gain Avg. Length (cm)±SE | Final gain Avg. Weight (g)±SE |
|---|---|---|---|---|---|
| 1. | Kolkata | 2.56 ± 0.01 | 1.59 ± 0.02 | 7.30 ± 1.92 | 8.60 ± 1.92 |
| 2. | Karnataka | 2.32 ± 0.02 | 1.54± 0.01 | 7.02 ± 1.42 | 7.64 ± 1.42 |
| 3. | Andhra Pradesh | 2.07 ± 0.06 | 1.53 ± 0.02 | 6.69 ± 1.40 | 6.96 ± 1.40 |

**Table 3. Water quality parameter range observed during the growth trials**

| SL. No. | Water Quality Parameters | Range | | | |
|---|---|---|---|---|---|
| | | West Bengal | Karnataka | Andhra Pradesh | Average |
| 1. | Water Temperature (°C) | 21-26 | 21-26 | 21-26 | 26.5 |
| 2. | Water pH | 6.75-8.40 | 6.86- 8.36 | 6.8-8.5 | 7.5 |
| 3. | Dissolved Oxygen (mg/l) | 5.9-8.1 | 6.9-8.3 | 6.5-8.9 | 8 |
| 4. | Electrical Conductivity (Siemens/meter) | 0.26-0.91 | 0.11-0.98 | 0.20-0.93 | 0.56 |
| 5. | Ammonia (mg/l) | 0.12-0.52 | 0.28-0.77 | 0.05-0.41 | 0.54 |
| 6. | Nitrate (mg/l) | 0.03-0.05 | 0.01-0.02 | 0.02-0.06 | 0.09 |
| 7. | Nitrite (mg/l) | 0.09-0.24 | 0.06-0.15 | 0.18-0.30 | 0.16 |

**Table 4. Number of polymorphic and monomorphic bands; and Percentage of polymorphic bands for the six selected primers for all the three stocks of *Labeo catla***

| Primers | Band Pattern | *Labeo catla* | | | Total (Mean ± SE) |
|---|---|---|---|---|---|
| | | Andhra Pradesh | Karnataka | West Bengal | |
| OPA-02 | P | 4 | 2 | 5 | 11 (3.67 ± 0.88) |
| | M | 1 | 3 | 0 | 4 (1.34 ± 0.86) |
| | %P | 80 | 40 | 100 | 220 (73.33 ± 17.64) |
| OPA-04 | P | 3 | 2 | 1 | 6 (2.00 ± 0.57) |
| | M | 1 | 2 | 3 | 6 (2.00 ± 0.57) |
| | %P | 75 | 50 | 25 | 150 (50.00 ± 14.43) |
| OPA-07 | P | 3 | 1 | 4 | 8 (2.67 ± 0.88) |
| | M | 2 | 4 | 1 | 7 (2.33 ± 0.88) |
| | %P | 60 | 20 | 80 | 160 (53.33 ± 17.63) |
| OPA-10 | P | 2 | 5 | 5 | 12 (4.00 ± 1.00) |
| | M | 3 | 0 | 0 | 3 (1.00 ± 1.00) |
| | %P | 40 | 100 | 100 | 240 (80.00 ± 20.00) |
| OPB-07 | P | 0 | 1 | 1 | 2 (0.67 ± 0.33) |
| | M | 3 | 2 | 2 | 7 (2.34 ± 0.33) |
| | %P | 0 | 33 | 33 | 66 (22.00 ± 11.00) |
| OPB-10 | P | 4 | 3 | 1 | 8 (2.67 ± 0.88) |
| | M | 1 | 2 | 4 | 7 (2.33 ± 0.88) |
| | %P | 80 | 60 | 25 | 165 (55.00 ± 16.07) |
| Average %P | | 55.83 ± 12.80 | 50.50 ± 11.38 | 60.55 ± 15.03 | |

*Where; P- polymorphic loci; M- monomorphic loci and %P- Percentage of Polymorphism*

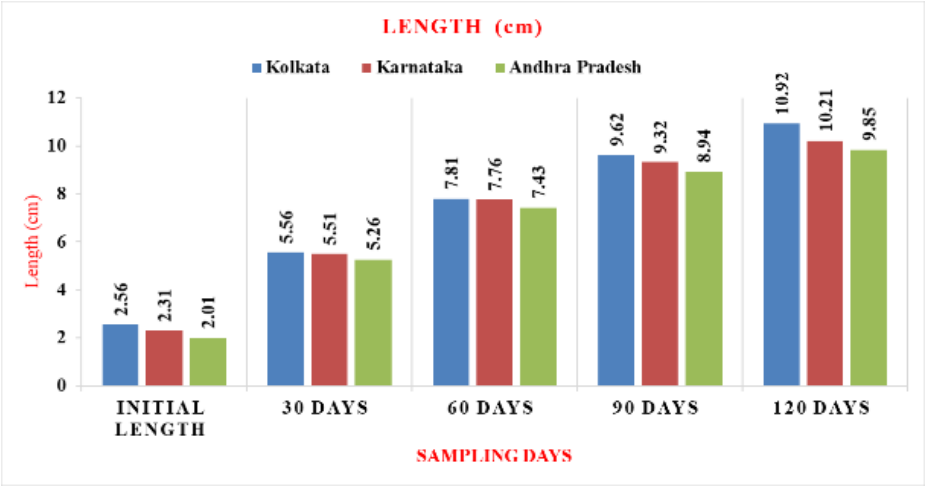



**Fig. 2. Mean Body Length (cm) attained by different stocks of *Labeo catla* at the end of growth trials**

has shown maximum polymorphism of 100 percent, followed by OPA-07 (80%), OPB-07 (33%), OPA-04 (25%), and OPB-10 (25%). Karnataka Stock, OPA-10 has shown the maximum percentage of polymorphism (100%) followed by OPB-10 (60%), OPA-04 (50%) OPA-02 (40%) OPA-07 (33%), while OPA-07 has shown minimum polymorphism of 20%. Andhra Pradesh stock, OPA-02 and OPB-10 have shown the maximum polymorphism of 80 percent followed by OPA-04 (75%), OPA-07 (60%), OPA-10(40%), and OPB-07 (0.0 %). The number of polymorphism bands, the number of monomorphism bands, and percentage of polymorphic bands for the selected six primers for all three stocks of Catla are represented by Table 4 and Fig. 4a, 4b, 4c, 4d, 4e, and 4f shows the agarose gel electrophoresis products generated with selected primers.

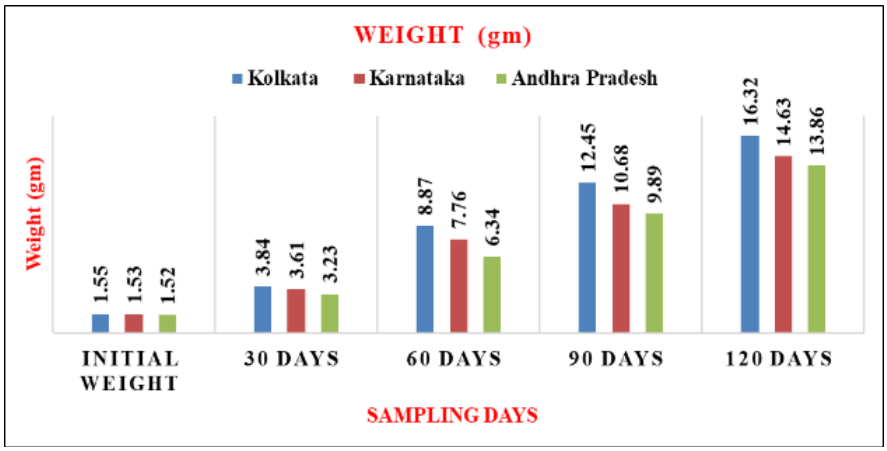



**Fig. 3. Mean Body Weight (g) attained by different stocks of *Labeo catla* at the end of rearing Period**

### 3.3 Nei's Heterozygosity and Percentage of Polymorphic loci

West Bengal stock showed the highest percentage of polymorphic loci (60.55%) followed by Andhra Pradesh (55.83%) and Karnataka (50.50%) stocks. West Bengal stock also showed highest heterozygosity (0.2017 ± 0.2164) followed by Andhra Pradesh (0.1957 ± 0.2260) and Karnataka (0.1398 ± 0.2057) stocks. The Nei's Heterozygosity, number of polymorphic loci, and percentage of polymorphic loci for the three stocks of Catla is presented in Table 5.

### 3.4 Genetic Variability and Genetic Diversity

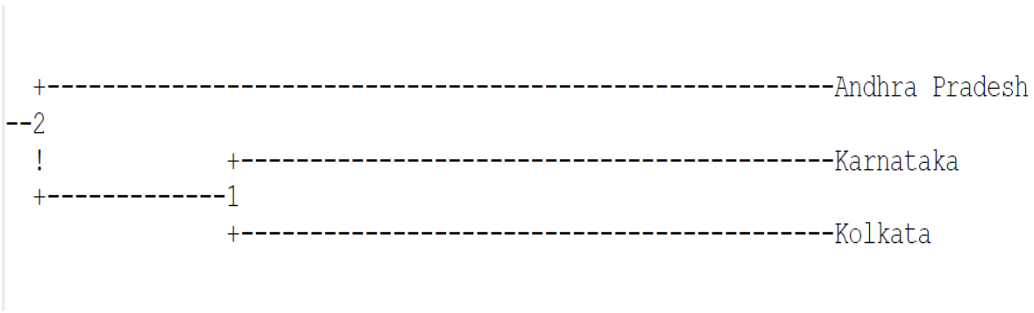
The Shannon's index was observed 0.3593 for West Bengal stock, 0.3097 for Karnataka and 0.1431 for Andhra Pradesh stocks (Table 6). Overall Shannon's diversity was 0.2701 for all three populations. Total genetic diversity (Ht) was found to be 0.4138, with the genetic diversity within populations (Hs) being 0.1791 and the overall average Gst value being 0.5672 with a gene flow (Nm) of 0.3816 (Table 7).

### 3.5 Genetic Identity and Genetic Distance

Karnataka and Andhra Pradesh stocks had the maximum genetic distance of 0.7135 and minimum Genetic identity of 0.4899, while the West Bengal and Karnataka stocks had 0.6268 and 0.4672 respectively. Similarly, Andhra Pradesh and West Bengal stocks had a genetic distance of 0.5127 and genetic identity of 0.5989. Genetic identity and genetic distance values observed for three different stocks of Catla are given in Table 8.

### 3.6 Dendrogram

The dendrogram for three stocks of catla is presented in Fig. 6, and the length between the clusters are given in Table 9. It indicated that the Andhra Pradesh stock and the other two stocks are differentiated into two clusters at a distance of 7.28218. Further, cluster one is divided into Karnataka and West Bengal stocks at a distance of 23.18265.



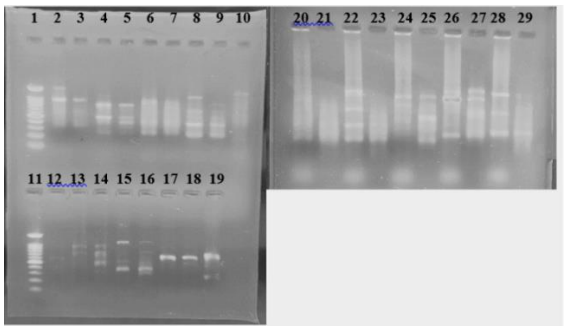



**Fig. 4a. RAPD profile of the maximum number of bands in West Bengal stock by OPA-02**

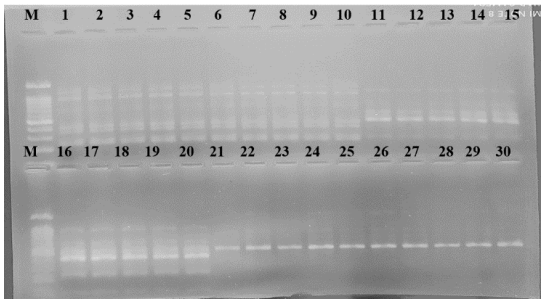



**Fig. 4b. RAPD profile of the minimum number of bands in West Bengal stock by OPA-04**

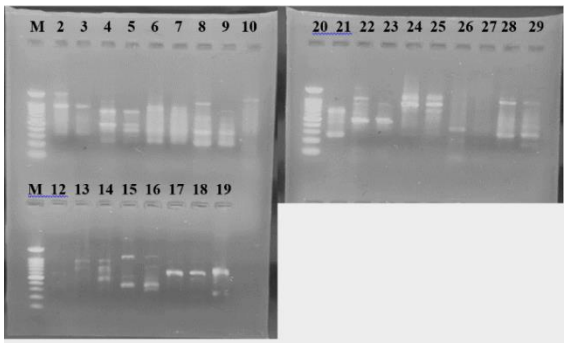



**Fig. 4c. RAPD profile of the maximum number of bands in Karnataka stock by OPA-10**

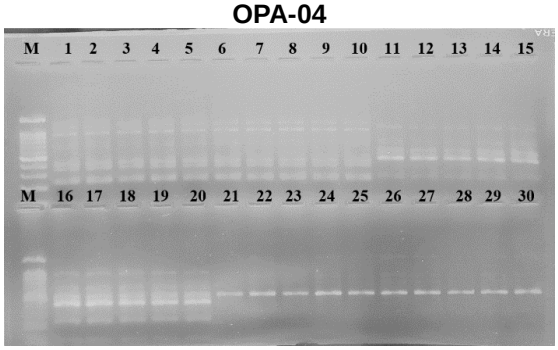



**Fig. 4d. RAPD profile of the minimum number of bands in Karnataka stock by OPA-07**

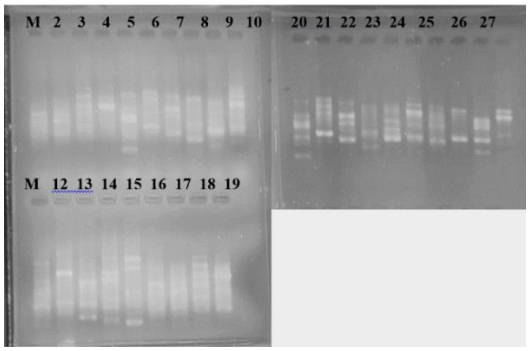



**Fig. 4e. RAPD profile of the maximum number of bands in Andhra Pradesh stock by OPA-02**

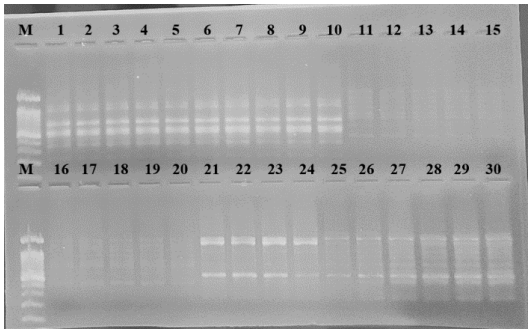



**Fig. 4f. RAPD profile of the minimum number of bands in Andhra Pradesh stock by OPB-07**

**Fig. 4. Dendrogram based on Nei's unbiased measures of Genetic Identity and Genetic Distance (Nei, 1978) in three different stocks of *Labeo catla***

**Table 5. Nei's Heterozygosity; Number of polymorphic loci; and Percentage of polymorphic loci for the three stocks of *Labeo catla***

| Sl. No. | Stock | Nei's Heterozygosity ± SD | No. of polymorphic loci | Percentage of polymorphism (%) |
|---|---|---|---|---|
| 1. | West Bengal | 0.2017 ± 0.2164 | 17 | 60.55 |
| 2. | Karnataka | 0.1398 ± 0.2057 | 14 | 50.50 |
| 3. | Andhra Pradesh | 0.1957 ± 0.2260 | 16 | 55.83 |

**Table 6. Genetic Variability and Genetic Diversity within the three populations as revealed by RAPD**

| Population | Na ± SD | Ne ± SD | He ± SD | I ± SD |
|---|---|---|---|---|
| West Bengal | 1.6889 ± 0.4682 | 1.4080 ± 0.3727 | 0.2395 ± 0.1966 | 0.3593 ± 0.2772 |
| Karnataka | 1.5111 ± 0.5055 | 1.3759 ± 0.4013 | 0.2119 ± 0.2169 | 0.3079 ± 0.3107 |
| Andhra Pradesh | 1.3111 ± 0.4982 | 1.1476 ± 0.2749 | 0.0922 ± 0.1576 | 0.1431 ± 0.2346 |
| Mean | 1.5037 ± 0.4906 | 1.3105 ± 0.3496 | 0.1812 ± 0.1903 | 0.2701 ± 0.2741 |

*Where; Na; Observed number of alleles; Ne; Effective number of alleles; He; Nei's Genetic Diversity; I; Shannon's Index; SD; Standard Deviation*

**Table 7. Diversity indices observed for *Labeo catla* populations in the present study**

| Markers | Total genetic diversity (Ht) | Intra-population genetic Diversity (Hs) | Inter-population genetic diversity (Gst) | Estimation of gene flow (Nm) |
|---|---|---|---|---|
| Random amplified polymorphic DNA | 0.4138 ± 0.0247 | 0.1791 ± 0.0160 | 0.5672 ± 0.00 | 0.3816 ± 0.00 |

**Table 8. Nei's unbiased measures of Genetic Identity and Genetic Distance (Nei, 1978) for the three stocks of *Labeo catla***

| Genetic identity / Genetic distance | Andhra Pradesh | Karnataka | Kolkata |
|---|---|---|---|
| **Andhra Pradesh** | **** | 0.4899**+** | 0.5989 |
| **Karnataka** | 0.7135* | **** | 0.6268* |
| **Kolkata** | 0.5127 | 0.4672**+** | **** |

*+Shows minimum identity/distance, *Shows maximum identity/distance*

**Table 9. Length between the clusters and different stocks**

| Between | And | Length |
|---|---|---|
| 2 | Andhra Pradesh | 30.46483 |
| 2 | 1 | 7.28218 |
| 1 | Karnataka | 23.18265 |
| 1 | Kolkata | 23.18265 |

### 3.7 Relationship between Polymorphism and Mean Body Weight

The relationship between the polymorphism and mean body weight attained by different stocks of catla was computed based on the correlation coefficient ($r^2$ value). The $r^2$ value between the percentage of polymorphism and mean body weight attained by different stocks of catla is noted as $r^2$: 0.00238; $P<0.05$).

## 4. DISCUSSION

The results of growth trials indicated that West Bengal stocks with a mean average body weight of 8.606 ± 1.923 g performed better in comparison with Karnataka (7.642 ± 1.422 g) and Andhra Pradesh (6.968 ± 1.40 g) stocks. The results of a one-way ANOVA demonstrated that there is no significant difference in mean body weight achieved by different stocks of catla, indicating that the minor differences in growth performance was perhaps due to genetic variation only. Since, all the stocks in the present study were subject to growth trials under the same environmental and feeding conditions, the only source of variation is possible with regard to the genetic variation that exists between the stocks. Popoola et al. (2014) had reported that nine primers (OPAD-09, OPAE-04, OPAE-05, OPAE-09, OPAF-07, OPAF- 08, OPAF-09, OPAF-11 and OPAF-12), out of the 20 primers tested, generated reproducible bands. The other 11 primers did not amplify or produced highly inconsistent amplification products from the same individual, and hence, the primers were excluded from further analysis. The lower polymorphism in the Karnataka and Andhra Pradesh stocks could be due to inbreeding, most likely connected to the maintenance of a lower

number of catla brood stock especially in the Andhra Pradesh breeding center. Basavaraju et al. (2014) reported that the eight primers were used of which OPF-12 exhibited high polymorphism (100%), while OPF-1 showed the lowest polymorphism (58.33%). The results indicated that the growth and polymorphism of different stocks showed a weak positive correlation. West Bengal stock showed a maximum heterozygosity (0.2017), followed by Andhra Pradesh (0.1957) and Karnataka (0.1398) stocks. However, Karnataka stock ranked second in terms of growth performance when compared to Andhra Pradesh stock, but West Bengal stock showed higher polymorphism than Andhra Pradesh stock. Thus, growth could be governed by a combination of polymorphism and heterozygosity. Further, Karnataka stock, though showed a lower polymorphism than West Bengal stock, the growth performance is poorer than that of the West Bengal stock which may be because of the lower heterozygosity of Karnataka stock (0.1398) than the West Bengal stock (0.2017). Satyaveer et al., (2023) reported that the Tamil Nadu Stock showed maximum heterozygosity (0.2395), followed by Karnataka Stock (0.2119), and Kolkata stock showed minimum heterozygosity (0.0922).

In the present study, Tamil Nadu Stock showed maximum heterozygosity (0.2395), followed by Karnataka Stock (0.2119) and Kolkata stock showed minimum heterozygosity (0.0922).

The survival rate of West Bengal stock is higher than Karnataka and Andhra Pradesh stock which perhaps could be linked to West Bengal stock showing higher heterozygosity than the other two stocks. The Karnataka and Andhra Pradesh stocks showing maximum genetic distance may be attributed to the two hatcheries containing superior economically important genes that may be absent in the other. West Bengal and Karnataka stocks having a minimum genetic distance may be credited to the possibility of cross-breeding between the two stocks before the collection of broodstock by the breeding center at Kolkata, which may also be the possible reason for the higher heterozygosity exhibited by West Bengal stock. The present study also showed that Shannon's index was 0.2957 for West Bengal Stock, 0.2505 for Andhra Pradesh Stock, and 0.2027 for Karnataka stock. Sharma et al. (2016) have reported that the genetic diversity and Shannon's Information Index values for the pond (0.16 and 0.24) are higher than that of cages at Kerwa Dam (0.15 and 0.22) and then from Narmada (0.12 and 0.18). The dendrogram constructed using the genetic distance data clearly differentiated different stocks of catla with Karnataka and West Bengal stocks clustering together indicating higher similarity. Since, Andhra Pradesh stock had formed a separate cluster, it could be concluded that a cross between West Bengal× Andhra Pradesh and Karnataka × Andhra Pradesh stocks would prove more beneficial. The genetic distance moderate between populations (typically in the range of 0.1 to 0.4 for genetic similarity) is considered ideal for crossbreeding. This suggests enough diversity to introduce beneficial genetic traits but not so much that the populations are highly divergent. The study indicates that RAPD could be employed to study the genetic variation in different stocks, and the technique can clearly differentiate stocks from diverse geographic locations representing different environmental conditions.

## 5. CONCLUSION

In conclusion, the RAPD approach proved to be a valid and effective tool for identifying and estimating genetic variability within and across the three Catla stocks. RAPD markers were effectively applied to detect intra and inter-bred polymorphism. The attributes of pace and economical attributes have favoured RAPD to be used in studies related to genetic variation in different stocks at a preliminary level where other advanced techniques fail to yield results; and in spite of reproducibility issues, the technique has been found to clearly differentiate various stocks belonging to diverse geographic locations representing varied environmental parameters. The unweighted pair group method with averages (UPGMA) dendrogram showed two clusters, the Kolkata and Karnataka populations forming one cluster and the other Andhra Pradesh populations in the second cluster. Genetic variation of *Labeo catla* is a useful trait for developing a good management strategy for maintaining the genetic quality of the species

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT etc) and text-to-image generators have been used during writing or editing of this manuscript.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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