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# Genetic variation among the wild and hatchery raised populations of *Labeo rohita* (Hamilton, 1822) revealed by RAPD markers

**R. Fayyaz[1*], N.A. Qureshi[2], P. Shakeela[3]**

[1]*Faculty of Fisheries and Wildlife, Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences, Lahore, Pakistan*

[2]*VC Government College, University for Women, Faisalabad, Pakistan*

**Key words:** Variation, Clustering, PCA, Wild, Hatchery, RAPAD, *Labeo rohita*.

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**Abstract**

The studies on genetic diversity of *Labeo rohita* by using molecular markers were carried out to investigate the genetic structure by RAPAD marker and the levels of polymorphism and similarity amongst the different groups of five populations of wild and farmed types. The samples were collected from different five locations as representatives of wild and hatchery raised populations. RAPAD data for Jaccard's coefficient by following the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) for Hierarchical Clustering of the similar groups on the basis of similarity amongst the genotypes and the dendrogram generated divided the randomly selected individuals of the five populations into three classes/clusters. The variance decomposition for the optimal classification values remained as, 52.11% for within class variation while 47.89% for the between class differences. The Principal Component Analysis (PCA) for grouping of the different genotypes from the different environmental conditions was done by Spearman Varimax rotation method for bi-plot generation of the co-occurrence of the same genotypes with similar genetic properties and specificity of different primers indicated clearly that the increase in the number of factors or components was correlated with the decrease in eigenvalues. The Kaiser Criterion based upon the eigenvalues greater than one, first two main factors accounted for 58.177% of cumulative variability.

* **Corresponding Author:** R. Fayyaz ✉ fayyazrasool@uvas.edu.pk

**Introduction**

The detection of genetic variation at the species, population and within population level is of great importance for sustainable aquaculture practices as well. Genetic variation at species level helps to identify the taxonomic units and to determine the species distinctiveness. Variation at the population level can provide an idea about different genetic classes, the genetic diversity among them and their evolutionary relationship with wild relatives. The genetic variability within population is extremely useful to gather the information on individual identity, breeding pattern, degree of relatedness and disturbances of genetic variations among them (Schierwater *et al.* 1994).

Genetic diversity is the existence of variants (alleles) of individual genes due to the change of the DNA sequence. The alleles of a particular gene may occur at different frequencies in different groups of individuals married (population) and genetic variation of a particular species because it is distributed both within populations (expressed as combinations of different alleles between individuals) and between populations (difference in occurrence and frequency between populations). A genetic sustainable fisheries implies one that does not result in loss of genetic diversity that cannot be accepted and/or change in unacceptable from the genetic composition of different populations or population systems. At present, it is unclear what level of loss/change can be considered as acceptable. Latest technological developments in the field of genetics have shown great potential for their application in fish conservation. Genetic variation can be directly assessed through controlled genetic markers. Signs may involve direct assessment of variation at the DNA level or through phenotypic expression, which can include proteins or morphological variants. Use of more than one marker can help enlarge the scope of utilization data. Information on the genetic structure of culturable fish species are useful to optimize the identification of populations, improvement of the population, improvement programs, performance management and sustainable conservation of genetic diversity (Dinesh *et al.* 1993; Garcia and Benzie 1995, Tassanakajon *et al.*1997). The four species of commercially important Indian carp, namely rohu, catla, mrigal and kalbasu belong to the family *Cyprinidae* and order *Cypriniformes* (Khanna, 1988; Talwar and Jhingran 1991). This classification is based largely on morphological characteristics. Karyotypic complements these tents seem to be very similar in terms of morphology and chromosome number (Manna and Khuda Bukhsh 1977; Zhang and Reddy 1991). These four species of carp do not intersect, but produce fertile artificial hybrids in any combination (Chaudhuri 1973; Bhowmick *et al.* 1981, Jhingran 1991). These species may have a common ancestral origin (Khanna 1988). However, information on genetic relationships and diversity of these species at the molecular level is not yet available. The partial cyto b is polymorphic and can be a potential marker to determining genetic stock structure. The 146 samples of *L. rohita* were collected from nine distant rivers; Satluj, Brahmaputra, Son, Chambal Mahanadi, Rapti, Chauka, Bhagirathi and Tons were analyzed. Sequencing of 307 bp of Cyto b gene revealed 35 haplotypes with haplotype diversity 0.751 and nucleotide diversity (p) 0.005. The within population variation accounted for 84.21% of total variation and 15.79% was found to among population. The total Fst value, 0.158 ($P<0.05$) was found to be significant. The study was for the examination of partial cytochrome b gene sequence of mitochondrial DNA for polymorphism and its suitability to determine the genetic differentiation in wild *Labeo rohita* (Luhariya *et al.*, 2012).

Much is yet to be done to find out the genetic divergence of these economically most important Indian carps especially in Pakistan. As mentioned, a number of methodologies are now available to proceed further in characterization and differentiation, to find out whether there exist different populations/stocks/strains among and within the species and the extent of genetic diversity and variation of these carps species. The research in this direction has been already initiated in India and is in progress in other countries including India. In

Pakistan, by carrying out such studies will help to determine the most fit and genetically sound candidates for the future breeding programs in the manmade hatcheries. The present study "Genetic variation among the wild and hatchery raised populations of *Labeo rohita* by using RAPD molecular markers was conducted detect the differences among five population of *L. rohita* from rivers and hatchery of Pakistan.

**Materials and methods**

The 50-samples for farmed *Labeo rohita* collected from UVAS-Fish Hatchery, C-block Ravi campus Pattoki district Kasur. The 50-samples from each site of almost having the same weight and other morphometric categories for wild *Labeo rohita* were collected from Trimu Barrage at the junction of Chenab and Jhelum Rivers near district Jhang, Taunsa Barrage at Indus River near tehsil Kot Adu district Muzaffar Garh, Qadirabad Barrage at Chenab River near district Mandi Bahuddin and Baloki barrage at Ravi River near tehsil Bhai Phero district Kasur (Figure-1). The experimental species from the above mentioned sites was collected and shifted to experimental laboratory by icing and was stored at -80°C.

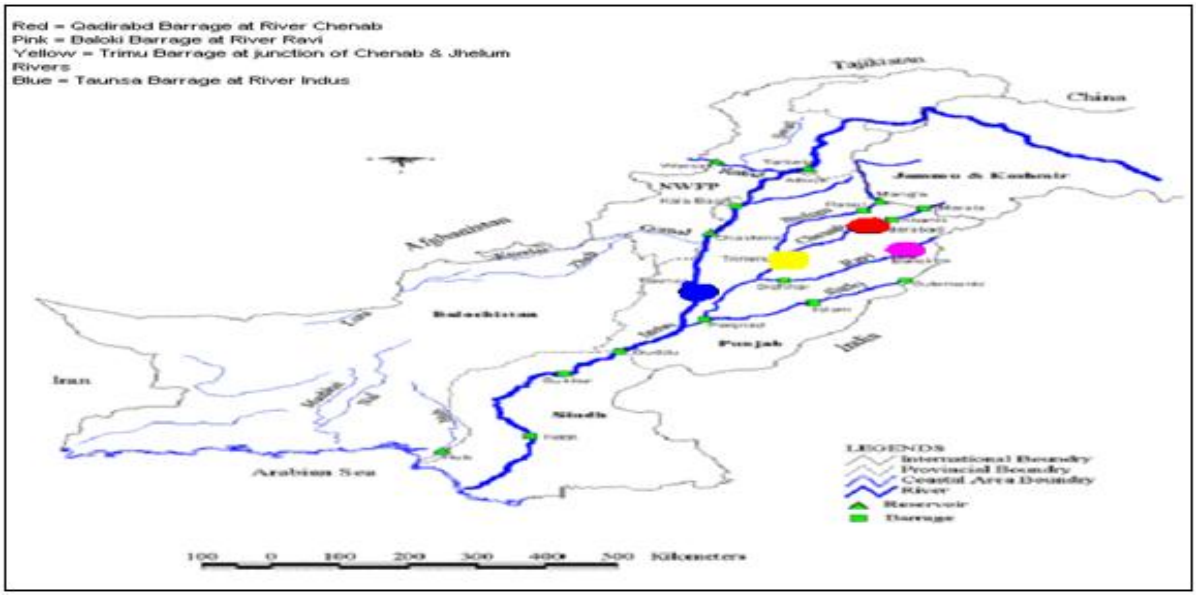


**Fig. 1.** The map showing the sampling sites at different Rivers of Punjab-Pakistan.

*DNA Extraction*

Total genomic DNA isolation was carried out from the stored fish samples using the procedure described by Lopera-Barrero *et al.* (2008). This procedure is based on the protocol given by (Aljanabi and Martinez, 1997) which was modified by the use of NaCl. In this procedure lysis buffer was used which carried 50mM tris which was taken from a stock of 1 M pH: 8 tris buffer, 50mM EDTA taken from a stock of 0.5 M pH: 8 , 100mM NaCl taken from a stock of 5 M NaCl and 1% SDS. From this lysis buffer working lysis buffer was prepared by adding 7µl of 200µgmL$^{-1}$ proteinase K. Stock solution of the proteinase K was prepared by preparing the buffer of 100mM Tris-base, 50mM EDTA, 500mM NaCl and then Proteinase K was added and dissolved at 200µgmL$^{-1}$. About 1g of the fish flesh or fin was taken in a 1.5 ml microfuge tube and homogenized in the 550µL lysis buffer, then 7µL of proteinase K buffer was added to the sample containing eppendrof microtube. After this the contents of the tube were incubated in thermoregulated water bath at 50°C for 12hrs. After this incubation 5 M NaCl amounting 600µL solutions was added and mixed thoroughly and then centrifuged for 10 minutes at 12000rpm. A fresh Eppendrof microtube was taken and supernatant was transferred into it with the help of micropipette. Then the DNA was precipitated by addition of 700 µL absolute cold ethanol. After mixing the contents of the tube, it was incubated at -20°C for 2 hrs. The tube

was then centrifuged 10 minutes at 12000rpm to obtain the pellet of the DNA. All the liquid was discarded and 300 µl 70% ethanol was added to remove salts. Then this washing with 70% ethanol was repeated and the pellet was dried by inverting the tube on a dry tissue paper. Air dried pellet of the DNA was dissolved in 80 µL TE buffer (10 mM tris and 1mM EDTA). To remove the RNA from these preparations 1µl of 30 µg/mL of RNAse was added and incubated at 37°C for one hour and then precipitated the DNA with 3.2 M sodium acetate and 2.5 volume absolute alcohol. The pellet was centrifuged, washed with 70% ethanol, dried and dissolved in 50 µL sterilized TE buffer.

*Quantification of DNA*

Purity of DNA was checked for quantification by using UV spectrophotometer (U-2800, Hitachi) and agarose gel electrophoresis. For this purpose Optical Density (OD) value at 260 nm and 280 nm were taken and calculations were made to determine the concentrations of the DNA samples. For the assessment of the integrity of the DNA samples all the samples were sequestered on 1% agarose gel prepared in 0.5X TAE buffer which was obtained from 50 X TAE stock solution prepared by dissolving 121 g tris base and 28.6 ml glacial acetic acid and 0.5 molar EDTA in water and raising its volume to 500 ml. The DNA samples were loaded into the gel after mixing with 10X DNA loading buffer with 0.21% bromophenol blue, 0.21% xylene cyanol FF, 0.2 molar EDTA and 50% glycerol.

*Primer Selection*

For this study 10mer (Operon) random primers were used to amplify polymorphic DNAs randomly. Twenty five primers designed by Gene Link Ltd., Hawthorne were used and the ten of these with most scorable bands were used further, the sequences of the used primers are given in table-1.

*PCR amplification of the Random Sequences from the fish samples*

With the help of the primers, polymerase chain reactions were devised. Each reaction was performed in 0.2 ml PCR tube and 25 µL reaction mixtures. To prepare this 25 µL reaction mix 2.5 µL 10x PCR buffer, 2 µL 1.6 mM $MgCl_2$ 2µL 10 nM primer, 2 µL 2.5 mM dNTPs, 0.3 µL 5 units/µL taq polymerase enzyme and 11.2 µL sterilized deionized double distilled water were mixed. In each reaction a negative control was also run using sterilized water as the template.

*Profile of the PCR Reaction*

PCR reaction was carried out in Personal Autorisieter Master cycler of the EPPENDORF, Germany. Each reaction profile was one cycle of 5 minute denaturation at 95°C and then 35 cycles of 1 minute at 95°C, 1 minute at 37°C and 2 minutes at 72°C and finally 20 minutes extension at 72°C. Then the machine was allowed to hold the reaction contents at 22°C.

*Analysis of the PCR products*

All the PCR products were analyzed by sequestering them on agarose gel. For this purpose 1.5% agarose gel was prepared in TAE buffer as described in section 3.6. The DNA samples were then loaded on the gel using the DNA loading buffer. Each gel was run with 100 base pair DNA ladder in the left and right lanes or only on one side. These gels were visualized in UV light and photographs were taken by gel documentation system (WEALTEC, Dolphin-DOC).

*Statistical analysis*

The XLSTAT 2012 version 1.02 of the computer software was used to analyze the RAPAD data for Jaccard's coefficient by following the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) for Hierarchical Clustering of the similar groups on the basis of similarity amongst the geneotypes and the dendrogram generated. The Principal Component Analysis (PCA) for grouping of the different genotypes from the different environmental conditions was done by Spearman Varimax rotation method for bi-plot generation of the co-occurrence of the same genotypes with similar genetic properties and specificity of different primers in the same program.

**Results**

The dendrogram developed by this method by the presented data of the scorable bands of the all amplified primers divided the randomly selected individuals of the five populations into three classes/clusters. All the randomly selected individuals of the five populations grouped themselves in the first class/cluster while a single sample designated as Indus2 from the population from River Indus collected from Taunsa Barrage represents the second class/cluster and in same way only single individual designated as Ravi2 collected from River Ravi from the Baloki Barrage represents the third class. The division of all the randomly selected five populations' representative *L. rohita* samples collected from different geographical locations in the three cluster was as follows; Hatch.1, Hatch.2, Hatch.3, Indus1, Indus3, Trimu1, Trimu2, Trimu3, Qad.1,Qad.2, Qad.3, Ravi1, and Ravi3 in first cluster/class while Indus2 in second and Ravi2 in the third class (Table 4). The results after analyzing the RAPAD data clearly indicated that these results clearly support the hypothesis of the present study.

**Table 1.** Sequences of the used primers.

| Sr. No. | Name of Primer | Sequence of the primer |
|---|---|---|
| 1 | OPB-1 | GTTTCGCTCC |
| 2 | OPB-3 | CATCCCCCTG |
| 3 | OPB-4 | GGACTGGAGT |
| 4 | OPB-5 | TGCGCCCTTC |
| 5 | OPB-7 | GGTGACGCAG |
| 6 | OPB-8 | GTCCACACGG |
| 7 | OPB-9 | TGGGGGACTC |
| 8 | OPB-10 | CTGCTGGGAC |
| 9 | OPC-19 | GTTGCCAGCC |
| 10 | OPD-4 | TCTGGTGAGG |

**Table 1.** Variance decomposition for the optimal classification of L. rohita on basis of RAPAD Data.

| | Absolute | Percent |
|---|---|---|
| Within-class | 0.308 | 52.11% |
| Between-classes | 0.283 | 47.89% |
| Total | 0.590 | 100.00% |

**Table 2.** Distances between the class centroids of *L. rohita* on basis of RAPAD Data.

| | **1** | **2** | **3** |
|---|---|---|---|
| 1 | 0 | 1.423 | 1.694 |
| 2 | 1.423 | 0 | 2.236 |
| 3 | 1.694 | 2.236 | 0 |

The variance decomposition for the optimal classification values remained as, 52.11% for within class variation while 47.89% for the between class differences (Table 1). The distance between the class/cluster centroids remained as; 1.423 for class one and two and 1.694 for class one and three while this distance was 2.236 for class two and three (Table 2). For these three classes/cluster Hatch.2 individual from the Hatchery population collected from Pattoki fish hatchery, Indus2, collected from Taunsa Barrage representative of the River Indus Population and Ravi2, from the Baloki Barrage the representative of

the River Ravi were identified as the central objects for class/cluster first, second and third, respectively. The clustering analysis showed the distances between the central objects of the different cluster/classes were 1.414 between class first and second central objects and 1.732 between the central objects of the class first and third while this distance between the central objects of the class second and third was 2.236 (Table 3). The results for the conclusion about the three classes with their values for within-class variance, minimum distance to centroids, average distance to centroid, and maximum distance to centroid for each class are given (Table 1).

**Table 3.** Distances between the central objects of *L. rohita* on basis of RAPAD Data.

| | 1 (Hatch.2) | 2 (Indus2) | 3 (Ravi2) |
|---|---|---|---|
| 1 (Hatch.2) | 0 | 1.414 | 1.732 |
| 2 (Indus2) | 1.414 | 0 | 2.236 |
| 3 (Ravi2) | 1.732 | 2.236 | 0 |

**Table 4.** Results by class of *L. rohita* on basis of RAPAD Data.

| Class | 1 | 2 | 3 |
|---|---|---|---|
| Objects | 13 | 1 | 1 |
| Sum of weights | 13 | 1 | 1 |
| Within-class variance | 0.308 | 0.000 | 0.000 |
| Minimum distance to centroid | 0.154 | 0.000 | 0.000 |
| Average distance to centroid | 0.393 | 0.000 | 0.000 |
| Maximum distance to centroid | 0.933 | 0.000 | 0.000 |
| | Hatch.1 | Indus2 | Ravi2 |
| | Hatch.2 | | |
| | Hatch.3 | | |
| | Indus1 | | |
| | Indus3 | | |
| | Trimu1 | | |
| | Trimu2 | | |
| | Trimu3 | | |
| | Qad.1 | | |
| | Qad.2 | | |
| | Qad.3 | | |
| | Ravi1 | | |
| | Ravi3 | | |

Spearman Varimax rotation method of Principal Component Analysis (PCA) was conducted to make conclusive results about the genetic relationships among the *L. rohita* collected samples from different geographical locations along with differentiation between and within the groups. The results obtained from the PCA indicated clearly that the increase in the number of factors or components was correlated with the decrease in eigenvalues (Table 5). The values in the table showed that its trend reached its maximum at level of second factor. In the same way according to the Kaiser Criterion based upon the eigenvalues greater than one, first two main factors accounted for 58.177% of cumulative variability. Therefore, we can assume after observing the results that the PCA grouped the tested variables or parameters of the fish RAPAD amplification data into two main components which all together accounted for 58.177% of the cumulative variation among the factors. The first group (F1) amongst the major two groups accounted for 33.327% of the cumulative variability while the second (F2) from these accounted for 24.850% of the cumulative variability (Table 5).

**Table 5.** Eigenvalues for PCA of *L. rohita*.

| | F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|---|
| Eigenvalue | 2.666 | 1.988 | 1.071 | 1.071 | 0.803 | 0.400 |
| Variability (%) | 33.327 | 24.850 | 13.393 | 13.393 | 10.035 | 5.003 |
| Cumulative % | 33.327 | 58.177 | 71.569 | 84.962 | 94.997 | 100.000 |

**Table 6.** Factor scores of *L. rohita* on basis of RAPAD Data.

| Observation | F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|---|
| Hatch.1 | 0.602 | 0.845 | 0.000 | -3.273 | -1.664 | -0.149 |
| Hatch.2 | 0.360 | 0.390 | 0.000 | 0.000 | 0.557 | 0.249 |
| Hatch.3 | 0.602 | 0.845 | 2.835 | 1.637 | -1.664 | -0.149 |
| Indus1 | 0.360 | 0.390 | 0.000 | 0.000 | 0.557 | 0.249 |
| Indus2 | 1.835 | -5.003 | 0.000 | 0.000 | -0.334 | -0.057 |
| Indus3 | 0.360 | 0.390 | 0.000 | 0.000 | 0.557 | 0.249 |
| Trimu1 | 0.360 | 0.390 | 0.000 | 0.000 | 0.557 | 0.249 |
| Trimu2 | 0.360 | 0.390 | 0.000 | 0.000 | 0.557 | 0.249 |
| Trimu3 | -1.129 | 0.088 | 0.000 | 0.000 | 0.782 | -2.260 |
| Qad.1 | 0.360 | 0.390 | 0.000 | 0.000 | 0.557 | 0.249 |
| Qad.2 | 0.602 | 0.845 | -2.835 | 1.637 | -1.664 | -0.149 |
| Qad.3 | 0.360 | 0.390 | 0.000 | 0.000 | 0.557 | 0.249 |
| Ravi1 | 0.360 | 0.390 | 0.000 | 0.000 | 0.557 | 0.249 |
| Ravi2 | -5.753 | -1.128 | 0.000 | 0.000 | -0.469 | 0.519 |
| Ravi3 | 0.360 | 0.390 | 0.000 | 0.000 | 0.557 | 0.249 |

**Table 7.** Contribution of the observations (%)of *L. rohita* on basis of RAPAD Data.

| | F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|---|
| Hatch.1 | 0.906 | 2.396 | 0.000 | 66.667 | 22.996 | 0.368 |
| Hatch.2 | 0.324 | 0.509 | 0.000 | 0.000 | 2.576 | 1.035 |
| Hatch.3 | 0.906 | 2.396 | 50.000 | 16.667 | 22.996 | 0.368 |
| Indus1 | 0.324 | 0.509 | 0.000 | 0.000 | 2.576 | 1.035 |
| Indus2 | 8.418 | 83.936 | 0.000 | 0.000 | 0.924 | 0.055 |
| Indus3 | 0.324 | 0.509 | 0.000 | 0.000 | 2.576 | 1.035 |
| Trimu1 | 0.324 | 0.509 | 0.000 | 0.000 | 2.576 | 1.035 |
| Trimu2 | 0.324 | 0.509 | 0.000 | 0.000 | 2.576 | 1.035 |
| Trimu3 | 3.188 | 0.026 | 0.000 | 0.000 | 5.083 | 85.037 |
| Qad.1 | 0.324 | 0.509 | 0.000 | 0.000 | 2.576 | 1.035 |
| Qad.2 | 0.906 | 2.396 | 50.000 | 16.667 | 22.996 | 0.368 |
| Qad.3 | 0.324 | 0.509 | 0.000 | 0.000 | 2.576 | 1.035 |
| Ravi1 | 0.324 | 0.509 | 0.000 | 0.000 | 2.576 | 1.035 |
| Ravi2 | 82.757 | 4.267 | 0.000 | 0.000 | 1.824 | 4.486 |
| Ravi3 | 0.324 | 0.509 | 0.000 | 0.000 | 2.576 | 1.035 |

The figure 4 showed the trends of most variable selected two factor in which the variables are the different primers towards their contribution for polymorphism amongst the randomly selected individuals of five populations. This trend divided the role of primers into five major variable group three groups towards the positive side and two groups towards the negative side. The figure 5 indicated the individuals of five populations taken as observations and divided into two major factors. It is clearly indicated that the representative individuals of five populations are genetically positively correlated with

each other with some exceptional case, like Trimu2 sample from Trimu Barrage the representative of the samples from junction of Jhelum and Chenab Rivers towards somewhat negative value and Ravi2 sample from the Baloki Barrage of the River Ravi towards the extreme negative value. The bi-plot analysis of the variables (primers) and observations (representative individuals of the five populations) was done, which indicated the level of similarity and differences among the five populations (Figure 6). The factor scores of the observations for similarity studies among the populations, the percentage contribution of each observation i.e. the individuals of the representative five populations in the genetic diversity and the squared cosines of the observations in which the values in bold correspond for each observation to the factor for which, the squared cosine is the largest are given in the tables 6, 7 and 8).

The varimax rotation is the alteration in axes in the PCA which increases the cumulative variability of the squared loadings. This is the orthogonal rotation which is used to show the influence or share of each individual. According to the Kaiser (1958) criterion, this may be the rotation which clears the individuals on such a level that "for each factor, high loadings (correlations) will result for a few variables; the rest will be near zero." The varimax rotation criterion maximizes the sum of the variances of the squared coefficients within each eigenvector, and the rotated axes remain orthogonal. The percentage of variability where the first two most common factors F1 and F2 have been rotated orthogonally and designated as D1 and D2. The cumulative variance is same as before rotation i.e. 58.177% with some variation in individual factor variation, which remained as 32.780% and 25.387% for D1 and D2, respectively (Table 9).

**Table 8.** Squared cosines of the observations of *L. rohita* on basis of RAPAD Data.

| | F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|---|
| Hatch.1 | 0.025 | 0.049 | 0.000 | 0.735 | 0.190 | 0.002 |
| Hatch.2 | 0.198 | 0.232 | 0.000 | 0.000 | 0.474 | 0.095 |
| Hatch.3 | 0.025 | 0.049 | 0.551 | 0.184 | 0.190 | 0.002 |
| Indus1 | 0.198 | 0.232 | 0.000 | 0.000 | 0.474 | 0.095 |
| Indus2 | 0.118 | 0.878 | 0.000 | 0.000 | 0.004 | 0.000 |
| Indus3 | 0.198 | 0.232 | 0.000 | 0.000 | 0.474 | 0.095 |
| Trimu1 | 0.198 | 0.232 | 0.000 | 0.000 | 0.474 | 0.095 |
| Trimu2 | 0.198 | 0.232 | 0.000 | 0.000 | 0.474 | 0.095 |
| Trimu3 | 0.182 | 0.001 | 0.000 | 0.000 | 0.087 | 0.729 |
| Qad.1 | 0.198 | 0.232 | 0.000 | 0.000 | 0.474 | 0.095 |
| Qad.2 | 0.025 | 0.049 | 0.551 | 0.184 | 0.190 | 0.002 |
| Qad.3 | 0.198 | 0.232 | 0.000 | 0.000 | 0.474 | 0.095 |
| Ravi1 | 0.198 | 0.232 | 0.000 | 0.000 | 0.474 | 0.095 |
| Ravi2 | 0.949 | 0.037 | 0.000 | 0.000 | 0.006 | 0.008 |
| Ravi3 | 0.198 | 0.232 | 0.000 | 0.000 | 0.474 | 0.095 |

*Values in bold correspond for each observation to the factor for which the squared cosine is the largest.*

**Table 9.** Percentage of variance after Varimax rotation of *L. rohita* on basis of RAPAD Data.

| | D1 | D2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|---|
| Variability (%) | 32.780 | 25.397 | 13.393 | 13.393 | 10.035 | 5.003 |
| Cumulative % | 32.780 | 58.177 | 71.569 | 84.962 | 94.997 | 100.000 |

**Table 10.** Squared cosines of the observations after Varimax rotation of *L. rohita* on basis of RAPAD Data.

| | D1 | D2 |
|---|---|---|
| Hatch.1 | 0.018 | 0.017 |
| Hatch.2 | 0.162 | 0.090 |
| Hatch.3 | 0.018 | 0.017 |
| Indus1 | 0.162 | 0.090 |
| Indus2 | 0.002 | 0.989 |
| Indus3 | 0.162 | 0.090 |
| Trimu1 | 0.162 | 0.090 |
| Trimu2 | 0.162 | 0.090 |
| Trimu3 | 0.069 | 0.009 |
| Qad.1 | 0.162 | 0.090 |
| Qad.2 | 0.018 | 0.017 |
| Qad.3 | 0.162 | 0.090 |
| Ravi1 | 0.162 | 0.090 |
| Ravi2 | 0.963 | 0.001 |
| Ravi3 | 0.162 | 0.090 |

The two factors D1 and D2 after varimax rotation are the different primers which showed trends towards their contribution for polymorphism amongst the randomly selected individuals of five populations. This trend divided the role of primers into five major variable groups, three groups towards the positive side and two groups towards the negative side (Figure 7). The individuals of five populations taken as observations and divided into two major factors after varimax rotation are presented (Figure 8). It is clearly indicated that the representative individuals of five populations are genetically positively correlated with each other with some exceptional case like Trimu2 sample from Trimu Barrage the representative of the samples from junction of Jhelum and Chenab Rivers towards somewhat negative value and Ravi2 sample from the Baloki Barrage of the River Ravi towards the extreme negative value. The bi-plot analysis of the variables (primers) and observations (representative individuals of the five populations), indicates the level of similarity and differences among the five populations which have been minimized after varimax rotation are presented (Figure 9). The squared cosines of the observations after varimax rotation are represented in bold correspond for each observation to the factor for which the squared cosine is the largest (Table 10).

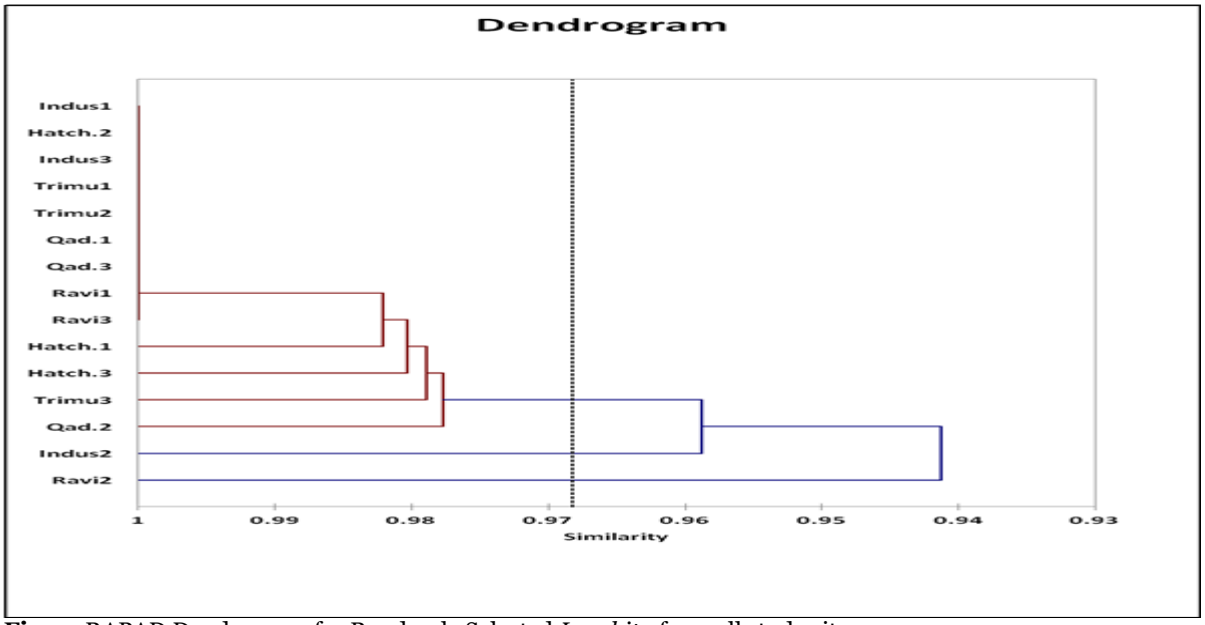


**Fig. 2.** RAPAD Dendrogram for Randomly Selected *L. rohita* from all study sites.

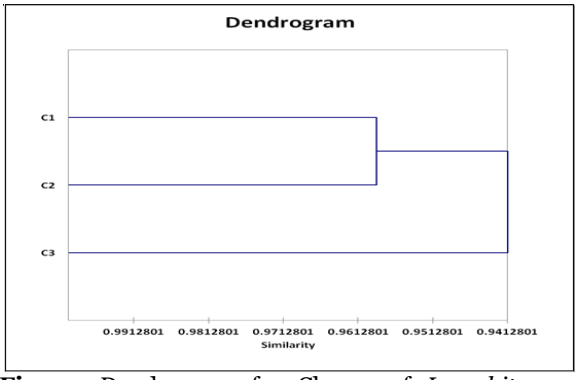


**Fig. 3.** Dendrogram for Classes of *L. rohita* on RAPAD data**.**

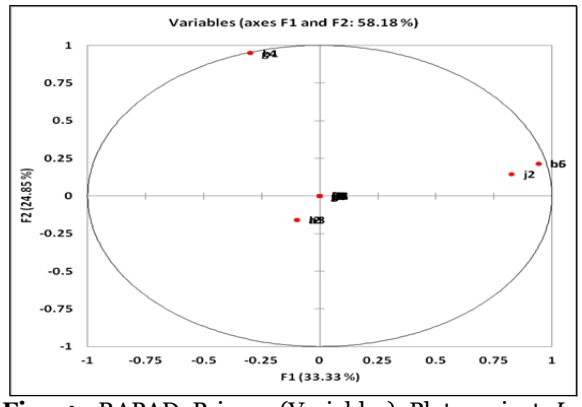


**Fig. 4.** RAPAD Primer (Variables) Plot against *L. rohita* samples. a = OPB-1, b = OPB-3, c = OPB-4, d = OPB-5, d = OPB-7, e = OPB-8, f = OPB-9, g = OPB10, h = OPC-19 and i = OPD-4.

**Discussion**

The dendrogram generated for the randomly selected individuals of the five populations divided the samples into three classes/clusters. It showed that there is only one individual in class two and three on the genetic basis and similarity with others (more that 90.00%), which clearly indicated that these results clearly support the hypothesis of the present study. These results are also in accordance with the Rahman *et al.* (2009) who generated Unweighted Pair Group Method with Averages dendrogram showing 2clusters, the population of River Halda appeared in one cluster, whereas all other natural populations including hatchery bunched in the second cluster. These results are also similar to the findings of Luhariya *et al.*, (2012) while studying the populations of *L. rohita* from nine distant rivers; Satluj, Brahmaputra, Son, Chambal Mahanadi, Rapti, Chauka, Bhagirathi and Tons by examination of partial cytochrome b gene sequence of mitocho The mtDNA cyto b sequences, analyzed in their study revealed moderate level of genetic differentiation in *L. rohita* wild population from nine different rivers and high within population variation. The variance decomposition for the optimal classification values remained as, 52.11% for within class variation while 47.89% for the between class differences.

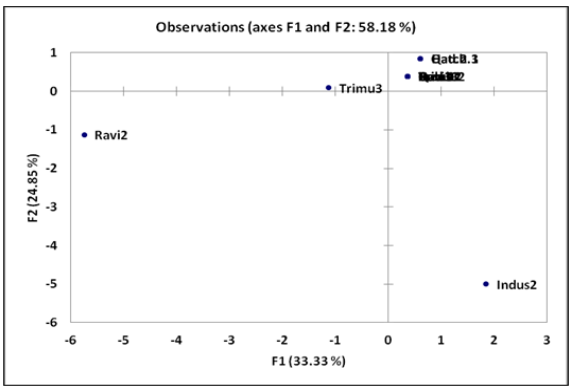


**Fig. 5.** *L. rohita* Samples (Observations) Plot by RAPAD Primer**.**

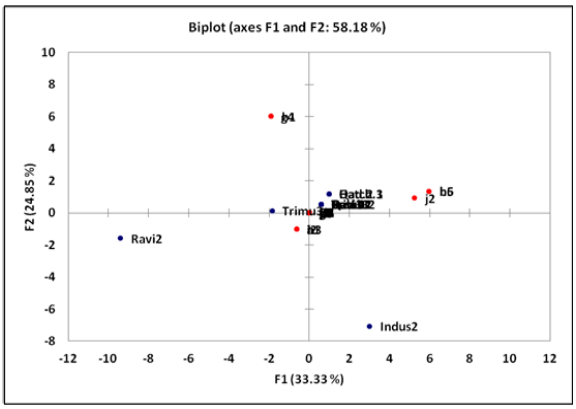


**Fig. 6.** *L. rohita* Samples and Primers (Variables and Observations) Bi-Plot by RAPAD Primer.

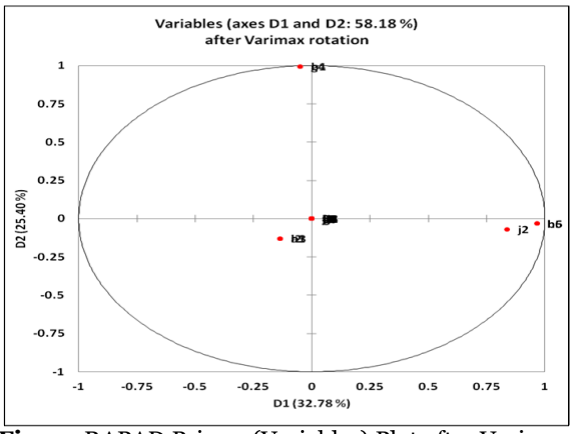


**Fig. 7.** RAPAD Primer (Variables) Plot after Varimax Rotation against *L. rohita* samples.

The distance between the class/cluster centroids remained as; 1.423 for class one and two and 1.694 for class one and three while this distance was 2.236 for class two and three. The results indicated that the representative individuals of five populations are genetically positively correlated with each other with some exceptional case like Trimu2 sample from Trimu Barrage the representative of the samples from junction of Jhelum and Chenab Rivers towards somewhat negative value and Ravi2 sample from the Baloki Barrage of the River Ravi towards the extreme negative value. This indicates that the some environmental impacts are likely showing their influence towards the genetic drifts. The bi-plot analysis of the variables (primers) and observations (representative individuals of the five populations), indicated the level of similarity and differences among the five populations. The cumulative variance was same as before rotation as 58.177% with some variation in individual factor variation which remained as 32.780% and 25.387% for D1 and D2, respectively. This trend divided the share of primers into five major variable groups, three groups towards the positive side and two groups towards the negative side. The results indicated that the individuals of five populations taken as observations and divided into two major factors after varimax rotation, these representative individuals of five populations are genetically positively correlated with each other with some exceptional case like Trimu2 sample from Trimu Barrage the representative of the samples from junction of Jhelum and Chenab Rivers towards somewhat negative value and Ravi2 sample from the Baloki Barrage of the River Ravi towards the extreme negative value. These findings are in accordance with the findings of Mostafa *et al.* (2009) who also found the initial level of genetic diversity (0.1238) when compared the populations of Kalibasu in Bangladesh. The results of present study showed that the hatchery population grouped with the other Riverine populations which indicated that the minor differences are likely due to environmental difference since the brood stock for hatchery population was derived from these wild sites. These results are also confirm the results of Mostafa *et al.* (2009) who suggested that minute difference in populations of Kalibasu were probably caused due to habitat degradation in many ways, which ultimately affects the genetic variation of Kalibaus. The within population similarity or among classes similarity in case of *L. rohita* clusters was, 98.00% among the individuals of 1$^{st}$class, 97.76% among the sub class of 1$^{st}$ class, 95.87% between the individuals of 2$^{nd}$ class while the individuals in class three showed 94.12% similarity. While these similarity values among the *C. mrigala* were remained as; 97.91% among individuals of 1$^{st}$ class, 97.70% between the individuals of 2$^{nd}$ class, 97.91% for individuals of class 3$^{rd}$ while 93.84% similarity of class 4$^{th}$. The very minute differences showed that all the populations of the same species are interrelated with each other in one way or the other. These results are similar to results of Alam and Islam (2005) that revealed a relatively low level of genetic variation at microsatellite loci within and between *Catla* populations, with genetic variation in the hatchery population were lower as compared to the River populations. The results of present study are in accordance with the results of Chauhan *et al.* (2007) who studied different populations of wild *C. mrigala* from different River basins and concluded that there existed low level of differentiation between the populations of the same species and this may be due to common ancestry and exchange of individuals among the River basins. The results are also confirmation of the results indicated in the study conducted by Dayu *et al.* (2007) on the genetic similarity amongst the wild populations of *Cyprinus carpio*. They concluded that there was a correlation between the clustering result and the geographical distribution. These results are also comparable with the results of Mohindra *et al.* (2007) on the genetic variability in three clariid species, *Clarias batrachus, C. gariepinus and C. macrocephalus* and the UPGMA phylogenetic tree revealed three distinct clusters: *C. batrachus; C. gariepinus* and *C. macrocephalus*. Aung *et al.* (2010) also sorted the genetic variation and similarities between the farmed and natural populations of *Cirrhina cirrhosis* and their results indicated that variation for one natural and two hatchery populations remained highly admixed.

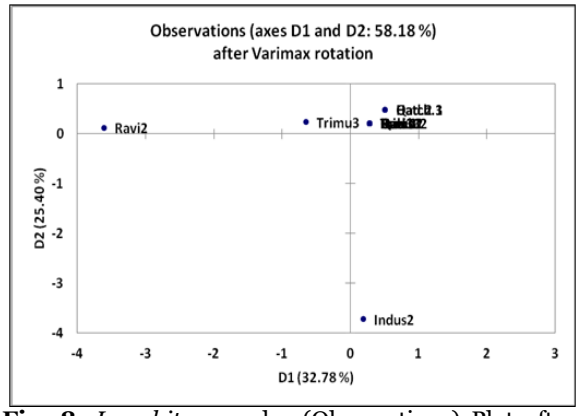


**Fig. 8.** *L. rohita* samples (Observations) Plot after Varimax Rotation.

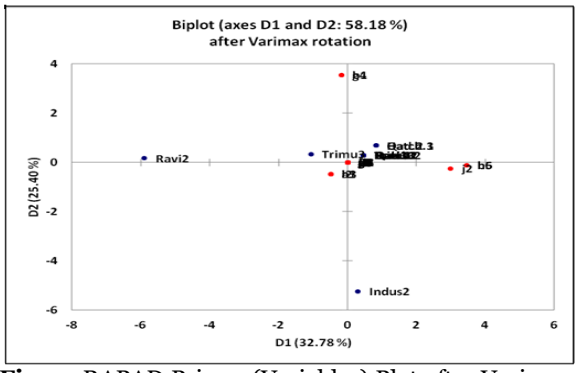


**Fig. 9.** RAPAD Primer (Variables) Plot after Varimax Rotation against *L. rohita* samples.

Knowledge of genetic structure of the major River populations and a typical hatchery population is helpful for management of the populations in order to maintain their genetic quality. In this study the results indicate good correspondence in the data analyses of morphometric parameters, and RAPAD molecular markers using various statistical techniques with the exception of the distinction of two individuals, Ravi 2 and Indus 2, which clearly indicated some environmental impacts, are likely influencing the genetic makeup within and between the local populations.

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