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Research Article

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Genetic Analysis of Cyprinid Species from Kashmir Valley Based on Random Amplified Polymorphic DNA

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ABSTRACT

The random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) was applied to analyze the genetic variation of the 3 populations of cyprinid species Cyprinus carpio var. communis, Cyprinus carpio var. specularis and Carassius carassius collected from different locations of Kashmir, India. Twenty five samples for each of the three species collected from different locations were used for this study. Of the 12 random primers used 8 generated the polymorphism and showed 82.23% polymorphism. The study showed RAPD marker efficiently helped in inter- and intra- species variation of the three species. The unweighted pair group method with averages (UPGMA) was used to construct dendrogram for RAPD which resulted in two clusters, one containing C. C. Communis and C. C. specularis and the other included the C. carassius species. The Phylogenetic analysis demonstrated that scale carp (Cyprinus carpio communis) is closest to mirror carp (Cyprinus carpio specularis) than from crucian carp (Crassius carassius).

Keywords: *Cyprinid, Genetic variation, RAPD, Polymorphism.*

INTRODUCTION

Common carp (*Cyprinus carpio* L.) belongs to the largest family of cyprinidae among freshwater teleosts[14]. Taxonomical analysis divides the currently existing common carp into three varieties: (1) the scale carp (*Cyprinus carpio var. communis)* (2) the mirror carp (*Cyprinus carpio var. specularis)* and (3) the leather carp *(Cyprinus carpio var. nudus)*, the later has not been reported in Kashmir, India[2]. Common carp was introduced in Kashmir in 1956 [5]. There is no published record of the introduction of C. *carassius* in Kashmir valley but it is considered that it got introduced accidentally together with introduction of *Cyprinus carpio*[15]. Common carp and crucian carp are locally called as punjabe gad and gang gad respectively.

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Molecular and genetic efforts were not carried out for understanding genetic relations of the species. Earlier studies remained restricted to morpho - taxonomical studies and are not ultimate tool for characterization of any species. Keeping in view that information on the genetic structure of fish species is useful for optimizing identification of stocks, stock enhancement, breeding programs, management for sustainable yield and preservation of genetic diversity[6,7]. Since the fishes have been introduced into the Kashmir without the knowledge about their phylogeny, it is imperative to study their genetic relation. The present study will be undertaken to study the genetic diversity in *Cyprinus carpio* & *Carassius carassius* species of Kashmir using RAPD with the following objective: To study the polymorphism using randomly amplified polymorphic DNA (RAPD) of two cyprinid species.

MATERIALS AND METHODS

Species and phenotypes studied. Species and phenotypes of family *Cyprinidae* used in this study and their source have been mentioned in Table 1.

Table 1: Species and phenotypes of family *Cyprinidae* used in this study and their source*

No.	Genus	Species	subspecies	Common name	Group name
1.	*Cyprinus*	*carpio*	*communis*	Scale carp	Common carp
2.	*Cyprinus*	*carpio*	*specularis*	Mirror carp	Common carp
3.	*Carassius*	*carassius*		Crucian carp	Golden carp

***** River Jhelum, Telbal Nallah, Dal lake, Anchar lake in Kashmir valley

DNA extraction. DNA was extracted from fine tissue (muscle) of each genus following the method described by Bardakci and Skibinski[3].

PCR Primers. In the present study, ten and twenty base long oligonucleiotide primers were used to initiate PCR amplifications. Primers were randomly selected on the basis of GC content and annealing temperature for RAPD amplification.

PCR amplification and agarose gel electrophoresis. In total 12 decamer RAPD primers were screened and only eight primers were found to efficiently generate the polymorphism. PCR was performed in a volume of 20 µl containing: 10x Taq DNA polymerase buffer, 2.25 mM MgCl2, 0.2 mM of dNTP mix, 0.36 µM of each primer (Sigma Aldrich, USA), 0.4ng genomic DNA, and 1 unit of Taq DNA polymerase (Sigma Aldrich, USA). A control PCR tube containing all components but no genomic DNA was run with each primer to check any contamination. DNA amplification was performed in Master Cycler Gradient (Eppendorf, Germany). After initial incubation for 5 min at 94^0c, the samples for enzymatic amplification were subjected to 45 repeats of the following thermal cycle: 1 min 94^0c, 1min at 36^0c and 1 min at 72^0c, and the final extension at 72^0c for 5 min. After amplification, the reaction products were subjected to electrophoresis in 1.5% agarose gels in 1x TAE buffer at 5 V/cm, stained with ethidium bromide and photographed under UV light with the help of Gel documentation system (Alpha-Innotech, USA). A Gene RularTM DNA Ladder Mix (Bangalore Genei, India) was used as the molecular standard. All the PCR results were tested for reproducibility by at least three times. Bands that did not show fidelity were eliminated.

Scoring and analysis of RAPDs. The DNA bands were scored for their presence (1) or absence (0) in the RAPD profiles. The band sharing index of similarity between the two

phenotypes of common carp was calculated using the formula: Bab =2 Nab/ (Na + Nb), where Nab is the number of common fragments observed in individuals a and b, and Na and Nb are the total number of fragments scored in a and b respectively (Lynch, 1990). The data matrix so generated was used for calculation of similarity matrix for all primers based on Jaccard's coefficients[12].

RESULTS

Standardization of DNA isolation protocol

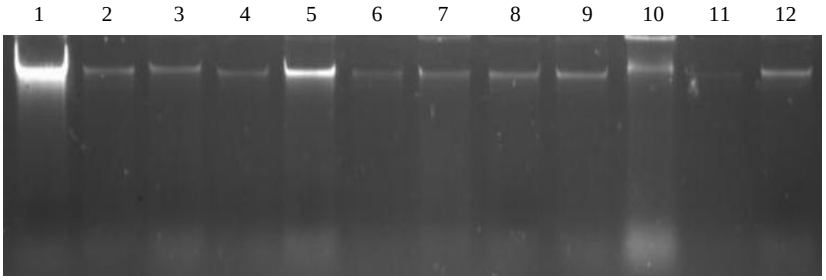

Fig. 1: Isolated DNA from cyprinid species resolved on 0.8% Agarose gel Lane 1 to Lane 11 is genomic DNA

RAPD Profiles

In this study, RAPD-PCR technique was used to assess the level of genetic diversity within and between the population of two cyprinid species. The primers with G + C content of above 60% resulted in better polymorphism (Fig 2,3,4).

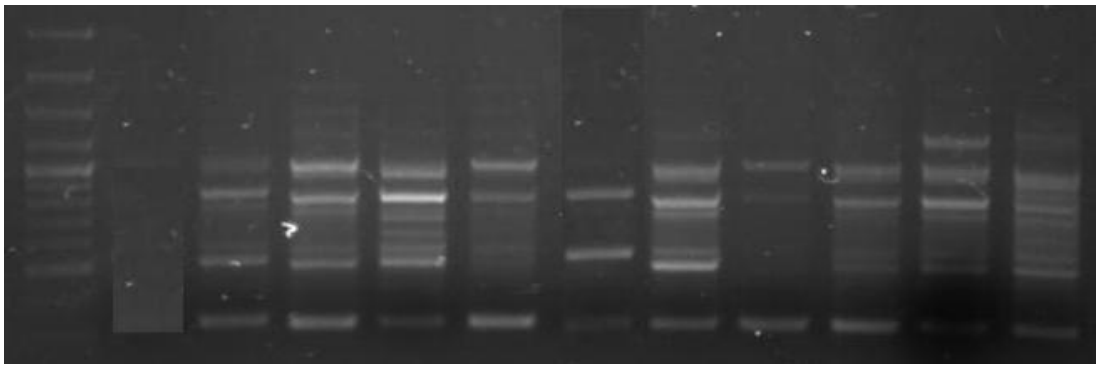

Fig. 2: 1.4% Agarose gel, primer S-177,100bp ladder,control,Sc-1,Sc-2, Sc-3,Sc-4, Sc-5,Sc-6,Sc-7,Sc-8,Sc-9,Sc-10

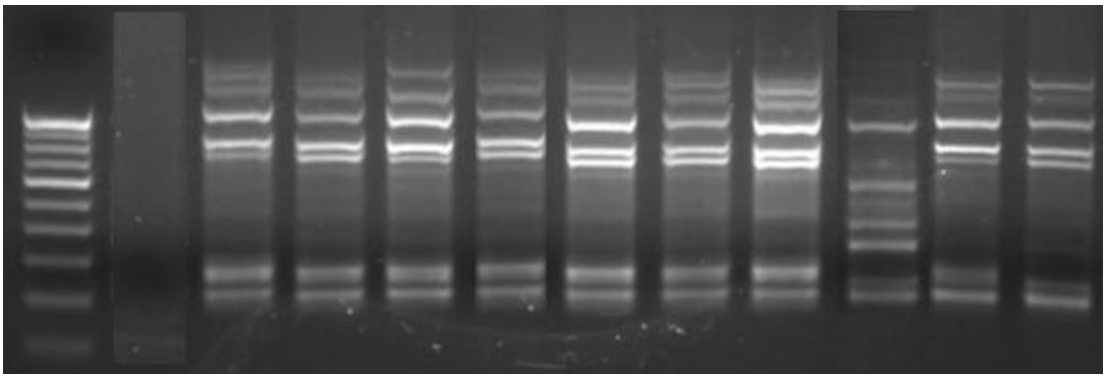

Fig. 3: 1.4% Agarose gel, primer S-159,100bp ladder, control, Mc-16, Mc-17, Mc-18,Mc-19,Mc-20,Mc-21,Mc-22,Mc-23,Mc-24,Mc-25

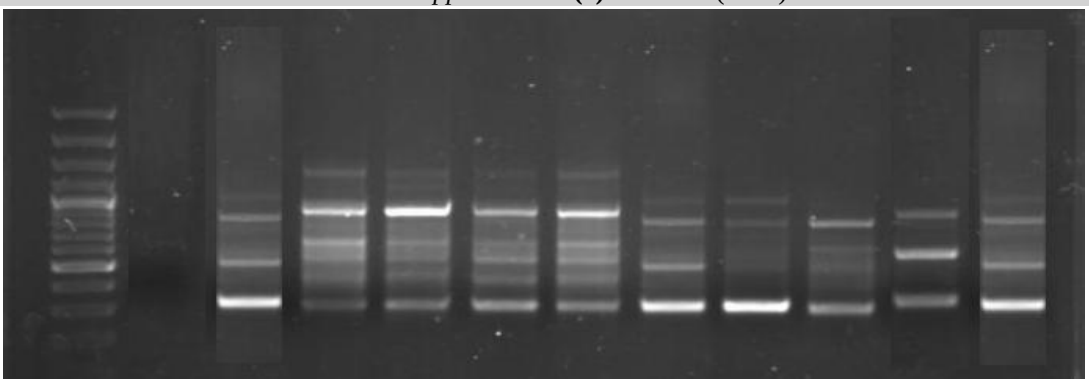

Fig. 4: 1.4% Agarose gel, primer S-111,100bp ladder, control, Cc-11,Cc-12, Cc-13,Cc-14,Cc-15,Cc-16,Cc-17,Cc-18,Cc-19,Cc-20

For further analysis, one way ANOVA was performed for all the three species and the result revealed the non- significant difference i.e. P>0.05 for both polymorphic and non-polymorphic banding pattern as shown in (Table 2, 3). Also the correlation between the polymorphic and non-polymorphic bands is positively correlated i.e r = 0.369. Statistically there is non-significant difference (P>0.05) as all values shown do not differ significantly in Table 4.

Table 2: Total number of bands (Mean ± SE)

Primer(N)	Species	Mean± SE
8	*Cyprinus carpio communis*	120.25 ± 41.36
8	*Cyprinus carpio specularis*	155.38 ± 50.81
8	*Carassius carassius*	145.75± 23.83
Mean		140.46 ± 38.66

Statistically there is non- significant difference as values do not differ significanly (P>0.05)

ANOVA Table

Source of variation	DF	SS	MS	F	P
Factor	2	5271	2636	1.630	0.220
Error	21	34021	1620		
Total	23	39292			

Table 3: Total number of polymorphic bands (Mean± SE)

Primer (N)	Species	Mean± SE
8	*Cyprinus carpio communis*	138.75 ± 68.16
8	*Cyprinus carpio specularis*	126.50 ± 26.00
8	*Carassius carassius*	110.50 ± 26.55
Mean		125.25 ± 40.23

Statistically there is non- significant difference as values do not differ significanly (P>0.05)

ANOVA table

Source of variation	DF	SS	MS	F	P
Factor	2	3211	1606	0.80	0.463
Error	21	42186	2009		
Total	23	45397			

Table 4: shows the correlation and P value between total no of bands and No of polymorphic bands

Primer(N)	Species	correlation	P value
8	*Cyprinus carpio communis*	0.663	0.073
8	*Cyprinus carpio specularis*	0.356	0.386
8	*Carassius carassius*	0.088	0.836

Statistically there is non- significant difference as all values shown in the table do not differ significanly (P>0.05).

For clustering analysis UPGMA dendrogram revealed that Crucian carp distributed in III cluster displayed maximum dissimilarity coefficient of 0.49 with other individuals, while, other two species i.e. Scale carp and Mirror carp clearly distributed in two clusters I & II respectively as shown in Fig. 4. The RAPD analysis proved to be an effective and efficacious technique to measure the magnitude of diversity and polymorphism of the species, as against the insufficient morphometric parameters.

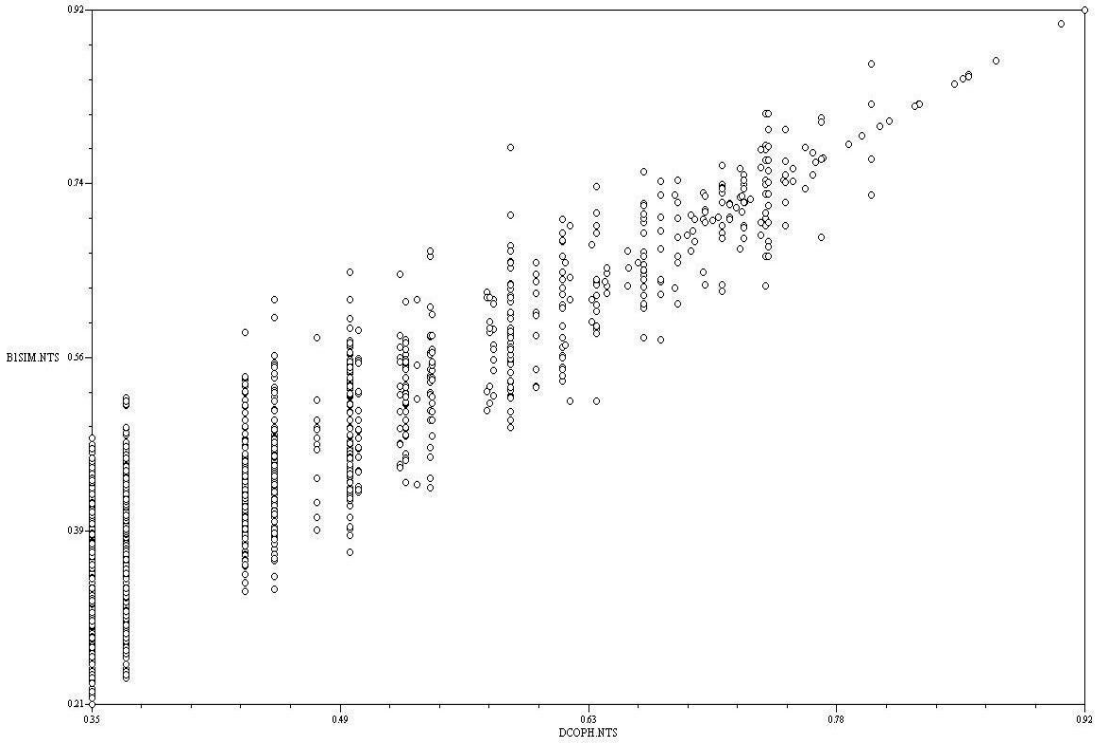

Fig. 4: Scatterplots: a coordinate graph of data points for RAPD analysis

DISCUSSION

Genomic DNA was isolated from each individual by modified protocol of Ahmad *et al* 2004 for molecular characterization of cyprinid species. The efficiency, speed and requirement of less expensive chemicals make the present method an attractive alternative to the existing methods of genomic DNA isolations in fishes. The protocol overcomes the need of liquid nitrogen, expensive lysis buffers and proteinase-k usually employed for fish DNA isolation. In terms of quantity (upto 174 µg/g of the muscle tissue) and quality ($A260_{/280}$=1.5 to 2.3), the present method has advantages over many prevailing fish DNA isolation protocols. By this protocol we were able to isolate DNA even from long preserved and refrigerated muscle tissue, which otherwise are recalcitrant to DNA isolation. The RAPD technique provides an efficient, simple and inexpensive method of generating molecular data. Further, it is highly polymorphic marker and does not require any prior knowledge of the genetic makeup of the organism[10]. In this study suitability and reliability of RAPD markers was assessed for understanding the phylogenetic relationships among and within the species of cyprinid species. In the present study of 12 decamer primers used to screen DNA samples, 8(66%) detected scorable polymorphism in banding pattern among all the 75 individuals. Eight selected primers generated a total of 3371 bands of which 3008 were polymorphic. The number of bands per individuals ranged from 4 to 12 and bands amplified ranged in size from 100-1400bp. The average number of bands

per primer ranged between 182 (S-131) and 497 (S-159) with a mean of 421.3. The proportion of polymorphic markers across the primers ranged between 79.75% and 97.96% with an average of 89.23%. Rahman *et al*. studied genetic variations of wild and hatchery populations of *Catla catla* by RAPD markers and found overall 54.55% polymorphism. Garg *et al.*[9] have also reported an analysis for RAPD to assess the extent of genetic diversity within and between three populations of the catfish, *Clarias batrachus* and obtained 72 scorable DNA fragments out of which 68 (86.66%) were polymorphic. We found that 89.23% of the loci in our study were polymorphic as compared to the 75% reported by Islam *et al.*[11] in *Catla catla*, 55.76% in *Oreochromis niloticus* , Zaeem and Ahmed, (2006), 64.98% in *Mystus vittatus* by Garg *et al*.[9], and 86.66% by Garg *et al*.[9], in assessment of genetic diversity of *Clarias batrachus.*

The UPGMA dendrogram obtained from the RAPD data clearly depicts the relationships among these three species. The highest interspecies genetic similarity was exhibited between *C.C. communis* and *C.C. specularis* and supports the hypothesis that these two cyprinids are closely related. The study was also done by Barman *et al.*[4] in Indian major carps. Similar to this present study, RAPD assay also been used to construct phylogenetic tree for resolving identification problems in Tilapia fishes by Bardakci & Skibinski[3].

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