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Studies on Variations Between Wild and Culture Population of Indian Major Carp *Catla catla* using Molecular Markers

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ABSTRACT: Genetic variations were studied in the three populations of most cultured Indian major carp, *Catla catla* liver tissue using Random Amplified Polymorphic DNA (RAPD) assay. Decamer primer, OPA-04 produced bands in the range of 0.9 - 2.5 Kb as is evident by the number and intensity of staining of bands. This study indicated that the genetic variations among the individuals of cultured population is very less as compared to wild and distant populations because of inbreeding depression. Hence, the chances of survival of wild and distant populations are more due to out-crossing within the gene pool to improve genome of carp fishes.

Keywords: Polymorphisms, DNA Profile, Inbreeding, Heterogeneity, Survival value.

INTRODUCTION

The long-term survival of fish population and the short-term fitness of species, genetic variations play important role as a natural gift because more than 99% animals are diploid. Hence, thereby chances of crossing over were more prone to reproduce recombinants. The ability of organisms to respond to selection and breeding programs and adjust to shifting environmental conditions comes from genetic variations. Genetic diversity assesses different types of genes or noncoding loci within population diversity. Both, heterozygosity and the overall number of alleles in a population influence its evolution and adaptation. Stressful environmental conditions lower genetic diversity, decrease population viability, and increase the likelihood of extinction (Martinez *et al.*, 2018). Genetic variations are similarly important in farmed populations allowing selective breeding and preventing loss of fitness due to inbreeding depression. Genetic variability can be view as two ways allelic diversity and heterozygosity. In small isolated populations, genetic variability can be substantially reduced through genetic drift and inbreeding resulting in the loss of alleles and decline in heterozygosity (Kumar *et al.*, 2020; Das *et al.*, 2021). Such reductions may result in decreased fitness and eventual extinction. To understand variations in fish and fisheries suitable molecular markers (Random Amplified Polymorphic DNA) were in use to discriminate species diversity and monitor genetic resources. PCR techniques are being employed in fish genetic study in an ever-growing number of methods, including RAPD (Randomly amplified polymorphic DNA) (Williams *et al.*, 1990; Welsh and McClelland 1990; Sagar *et al.*, 2020), microsatellite DNA (repeats of 1-4 nucleotide bases, typically with a total length of less than 300 bp) (Ahmed and Abbas 2018; Faroque *et al.*, 2021) and AFLP (amplified fragment length polymorphisms). All these methods have pros and cons when it comes to analyse fish genetic variability (Ferguson and Danzmann 1998; Ward, 2000).

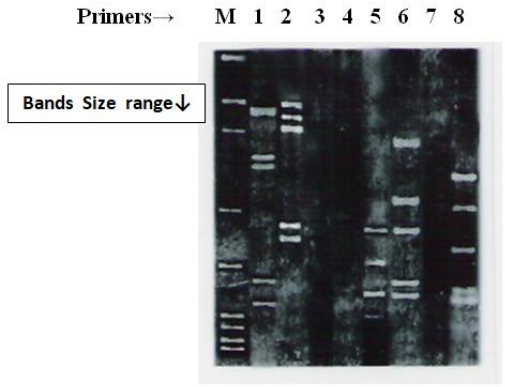
The present investigations were on the Indian major carp, *Catla catla* (collected from three different locations) with the above mention considerations in mind. This species constitute an important component of freshwater fishery and is mainstay in aquaculture production. Use of limited number of brood stock for breeding and seed production and continued mating between siblings may be responsible for shrinking of genetic base in most of the fish producing establishments and hatcheries. These result in low survival and poor growth rates, susceptibility to various diseases and abnormalities, and so on. Because of inbreeding, fatal genes become dominant due to homozygosity, resulting in death. Wild stocks usually outperform farmed stocks in terms of growth and survival, showing that the current techniques used for hatchery seed production are not proper. As a result, it becomes critical to do research on genetic improvement of this carp using various methods. Molecular study of the carp species is an adequate step in this direction. However, fairly limited genetic information is known on research relating to genetic variation in wild populations of Catla using random amplified polymorphic DNA (RAPD). During the present study, RAPD analysis method was used to determine the genetic polymorphisms and genetic variations in the three populations of *C. catla* at intra-population and inter-population (Intra-specific) level.

MATERIAL AND METHODS

Freshwater fish *Catla catla*, belonging to the family Cyprinidae and order Cypriniformes has used to study genetic polymorphism. To avoid ontogenic problems, fishes of the same size and approximately of the same weight used. Three populations of *C. catla i.e.* Population A from National Fish Seed Farm Jyotisar near Kurukshetra (cultured fish), Population B from various ponds and ditches of District Kurukshetra (Haryana) (Wild population) and Population C from various ponds and ditches of Howra, West Bengal (distant wild population) were analyzed in the present study. Fresh specimens of *C. catla* were collected and proper care under taken with respect to fish feed, dissolved O_2 content and water temperature so that the fish thrived well. For the purpose of RAPD analysis, liver tissue from all the three populations of the carp used. All the tissue samples were stored at –20ºC. High molecular weight DNA extracted from liver tissue of Catla collected from populations A, B and C using the Phenol-Chloroform Extraction Method. The intact DNA on gel without any smear showed that the quality of DNA was very good and DNA was quantified using absorbance at 260 nm. A significantly larger amount of genomic DNA extracted and found appropriate for amplification in polymerase chain reaction (PCR) for carrying out RAPD analysis. Agarose Gel Electrophoresis carried out for analysis. In RAPD-PCR, each fragment, regardless of primer, treated as independent locus. All gels scored manually and independently. The fish individuals' RAPD patterns compared both within and between the populations. Gels genetically analyzed by following the method explained by Pasteur *et al.* (1988). The observed data was analysed using the Rothe (1994) method. One-way ANOVA (Analysis of Variance) was applied to assess genetic variation at intra-specific (between populations).

RESULTS

During the present study, out of the eight decamer primer used, OPA-04 primer produced the best results and hence, used for further study in the carp (Fig. 1). It revealed five bands in each fish liver tissue. Total 8 bands of amplicons were observed in all the three populations *i.e.* A, B and C on agarose gel with size ranged from 0.90 Kb to 2.50 Kb. (Fig. 2). Polymorphism was very low (20.00%) in population A as four of the five bands were found to be monomorphic. In population B, two of the five bands of sizes 2.3 Kb and 2.5 Kb were monomorphic and three bands having size 1.4 Kb, 1.6 Kb and 2.1 Kb were polymorphic. Out of the total seven bands in population C, only one band of size 2.3 Kb was monomorphic while the rest with sizes 0.9 Kb, 1.2 Kb, 1.5 Kb, 1.6 Kb, 2.1 Kb and 2.5 Kb observed to be polymorphic. Hence, Population C was highly polymorphic (85.71%) as compared to the other two populations. 60.00% polymorphism found in population B. No unique band observed in any of the populations of this species. In all, 87.50% polymorphism shown by this fish species.



Sr. No.	Primers
1	OPA-02
2	OPA-04
3	OPA-05
4	OPF-17
5	OPC-02
6	OPC-11
7	OPA-03
8	OPA-16

Fig. 1. RAPD profile generated with eight decamer primers in *Catla catla*. (Lane M: 100-bp DNA ladder).

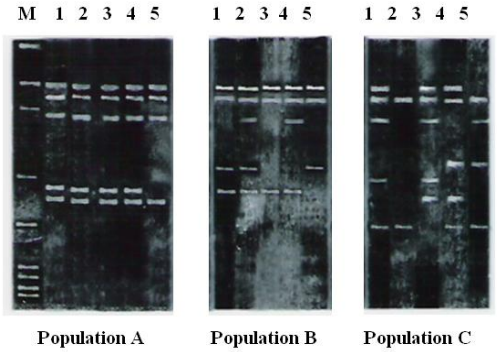


Fig. 2. RAPD profile generated with OPA-04 primer in five fishes of each of the populations A, B & C of the carp species, *Catla catla*. (Lane M: 100-bp DNA ladder).

One-way ANOVA test also showed significant variations in this species (Table 1 and 2). The genetic interpretation of RAPD profile, Similarity index within and between populations using the OPA-04 primer, the data obtained, depending on the presence and absence of bands on agarose gel of PCR product, and this data used to generate genetic similarity coefficients within and between the populations of Catla.

The values of pairwise genetic similarity coefficients of the five individuals of population A were 1.00 with an average of 1.00 while in population B, pairwise similarities among five individuals ranged from 0.571 to 0.888 with an average similarity of 0.783. In population C, pairwise similarity coefficients ranged from 0.200 to 0.666 with an average of 0.527. Similarity coefficient among three populations of this species observed to be 0.615. (Table 3)

Table 1: The number of RAPD loci in each population detected by primer, OPA-04.

Primer	Fish Species	Populations	RAPD Loci				
			Number of Loci	Monomorphic Loci	Polymorphic Loci	Unique Loci	Polymorphic Loci %
OPA-04	*C. catla*	A	5	4	1	0	20.00
		B	5	2	3	0	60.00
		C	7	1	6	0	85.71
		Total	8	1	7	0	87.50

Table 2: One-way ANOVA results showing variations at intra-specific level.

Sr. No.	Source of Variation	F-values	Results
1	Between Populations (*Catla catla*)	**F** = 5.66 ν_1=2, ν_2=6	Significant at 5 %

Table 3: Genetic pairwise similarity coefficients within and between the populations of Indian major carp, *Catla catla*.

Populations Pairs of 5 individuals	Population A	Population B	Population C
1	1	0.888	0.571
2	1	0.857	0.200
3	1	0.750	0.600
4	1	0.857	0.666
5	1	0.750	0.285
6	1	0.888	0.571
7	1	0.750	0.666
8	1	0.857	0.600
9	1	0.666	0.444
10	1	0.571	0.666
Similarity Within Population	1	0.783	0.527
Similarity Between Populations	0.615		

P. = Population; **Pairs** = all possible pairs between five individuals *i.e.* 10.

DISCUSSION

There is growing evidence that nuclear DNA variation in fishes, between subspecies, or between populations of the species can be detected using the RAPD method (Williams *et al.*, 1990; Mamuris *et al.*, 1998b, 1999) and between different species (Mcglashan and Hughes, 2001; Barman *et al*., 2003; Prasad, 2014). The technical simplicity of RAPD method and prevalence of polymorphism in fishes suggested that RAPDs could provide more data that are accurate on fisheries genetics (Ferguson and Danzmann 1998). It produces a significant number of polymorphic DNA bands within and between different fish species. During the present investigation, five primers *i.e.* OPA-02, OPA-04, OPC-02, OPC-11 and OPA-16 generated location-specific and species-specific PCR amplicons in the size range of 0.9 kbp – 2.5 kbp, in *Catla catla*. Primers OPC-02 and OPC-11 produced 5 and 4 PCR products. With the same primers, Bielawski and Pumo (1997) could get 12 and 13 PCR products in Atlantic coast striped bass (*Morone soxatilis*) and Barman *et al.* (2003) could get 3 to 10 bands between 0.25 Kbp to 1.50 Kbp in size in rohu, kalbasu, catla and mrigala. During this study, primers OPA-02 and OPA-16 produced 5 PCR products. But Mamuris *et al.* (1999) could get a large number of bands (154) with the same primers (OPA-02 & OPA-16) used in the RAPD analysis of striped red mullet (*Mullus surmuletus* L.). Primers OPF-17, OPA-03 and OPA-05 did not yield any results during the present study. In Liu et al.'s (1999) investigation of genetic variation in catfish, 75 high and medium quality primers produced 462 polymorphic bands (size range-

0.2kbp to 1.5kbp), with an average of 6.1 bands per primer in this fish species.

Presently, Primer OPA-04 generated five to seven bands in different populations of the carp species. Mamuris *et al*. (1999) could get a large number of bands per primer (14-22) in striped red mullet (*Mullus surmuletus* L.). This difference is due to different fish species analyzed by different workers. RAPD profile of four species of Indian major carps studied (Barman *et al.,* 2003) generated 900 scorable bands with an average ranging between 6.3 to 7.4 bands per primer but this average was low (3.6 to 4.8) in the carp species under present investigation. This difference can be due to the differences in the sites of collection. In the present investigation, 87.50% polymorphic bands exhibited between the populations of *C. catla*. In comparison to this, the polymorphic bands percentage ranged from 56.2% to 64.9%, averaging 59.4% in each sample of European sea bass (*Dicentrarchus labrax*) analyzed (Caccone *et al*., 1997). Mamuris *et al*. (1999) could get 73 polymorphic bands out of total 154 bands (47.4%), and the rest were monomorphic, constantly present in all individuals. Present study also depicted one monomorphic band in *C. catla.* Thus, the level of polymorphism was more in the carp species analyzed during the present investigation as compared to that in European sea bass and red mullets earlier studied (Caccone *et al*., 1997; Mamuris *et al.,* 1999). Identification of unique bands (bands present only in certain individuals *i.e*. individual specific) is an important feature of RAPD to study variation within populations and to identify the stock structure. During the present investigations, no unique band observed in *C. catla.* Hence, it is clear that unique bands are present in low number and are individual specific. Our observations supported the findings of Caccone *et al.* (1997) who could identify only three unique bands out of 107 bands analyzed in the European sea bass (*Dicentrarchus labrax*).

RAPD based genetic variation depicts that the levels of intra-population variation may vary with the site of sample collection and with the type of species. During the present investigations, the intra-population variation was found to be absent or very low in the hatchery-obtained population A (genetic similarity = 1.00) of all the three fish species. The present observations supported the findings of Bardakci and Skibinski (1994) who also obtained the highest value of within-population similarity (s) in *O. mossambicus* and the lowest for *O. niloticus i.e.* intra-population variation was very low as was in case of population A of the carp species under the present study. Bardakci and Skibinski (1994) further indicated higher similarities between subspecies of *O. niloticus* (except between *O. n. vulcani* and *O. n. niloticus*) than those between the species of *Oreochromis*. It observed that levels of intra-population variations were low because most of the subspecies propagated from relatively small numbers of individuals. This type of low level of intra-population variability has seen during the present analysis in the specimens derived from hatchery (Population A). Population C exhibited more variation between individuals as compared to population B in the above said carp species (Table 3). One-way ANOVA test depicted significant variation between the populations of *C. catla* (F=5.66, Significant at 5%). Elo *et al.* (1997) assessed the heterozygosities of nonanadromous salmon and brown trout using RAPD data to be approximately 0% and 5%, respectively. The low degree of genetic variation was attributed to genetic drift and inbreeding.

During the present investigations, the population B, collected from the wild ponds and ditches of Kurukshetra, Haryana, exhibited low level of genetic variations as compared to population C of the same carp species collected from Howra. This is because these latter populations were being located at a considerable distance from each other. Barman *et al.* (2003), while studying genetic variation among mrigal, kalbasu, rohu and catla using RAPD markers earlier obtained similar results. They could observe low levels of genetic variation within-species in cultivated kalbasu and rohu stocks. They further maintained that this condition might be due to their repeated propagation and maintenance for long period with limited number of individuals sampled from wild. According to them, this was an indication of possible high rate of inbreeding in Indian major carps, as was also observed earlier (Eknath and Doyle 1990) using the criteria of effective population size. They further claimed that this condition caused by their long-term, repetitive propagation and maintenance with a small number of wild individuals sampled. They claimed that this was a sign of a potential high rate of inbreeding in Indian main carps, which was also noted (Eknath and Doyle 1990) using the effective population size criterion.

CONCLUSIONS

The presently obtained results confirm these assertions. Relatively higher levels of intraspecific genetic variation exhibited in *C. catla* might be due to comparatively lower rate of propagation. Because of its somewhat reduced rate of propagation, *C. catla* showed substantially larger levels of intraspecific genetic diversity. During the present investigations, we observed relatively higher levels of intra-specific genetic variation in catla (87.5 % polymorphism). This might be due to comparatively lower rate of propagation in catla. We found comparatively greater levels of intra-specific genetic variation in catla (87.5%) during the current investigations. This could be because catla has a relatively slower rate of propagation. The results of the current study found that the gene pool of farmed variants is only a fraction of the gene pool of the wild forms of the Indian major carp, *C. catla*. The genetic heterogeneity among cultured populations of various carp species is lower than that within wild forms. As a result, frequent outcrossing of cultivated populations with wild populations has a better chance of producing sturdier, highly nutritious, and more productive varieties of this carp species than selective breeding followed by

inbreeding among cultivated populations. But, because of overexploitation, pollution, and inbreeding depression; the wild population of Catla, is also decreasing in Indian rivers. Hence, when fish populations are on the verge of extinction, artificial breeding programs should be used to improve wild populations in fisheries management. These tactics may have increased stock sizes while retaining genetic variety and also, reducing the risk of local extinction (Neff *et al*., 2011).

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