Isozyme Profile of Esterases in Backcrosses of *Catla Catla* (Ham.) and *Labeo Rohita* (Ham.)

By Subodh Kumar Tripathy & Niranjan Sarangi

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**Abstract** - Under captive conditions of ponds, reservoirs and other stagnant aquatic bodies in Indian peninsula, the major carps viz. *Catla catla* (Ham) and *Labeo rohita* (Ham) have their unique and equal importance contributing lion shares to freshwater aquaculture. With an intention to improve the genetic architecture of these carps, their backcross generations were developed in Central Agricultural Research Institute, Port Blair, South Andaman to establish some of the desired morphometric characters such as small and narrow head of rohu as well as deep, broad body of catla in their backcross progenies using the technique of induce breeding. To reveal the hereditary trend, the esterase profile was developed and the isozyme marker indicated more genetic proximity of backcross progenies with rohu than catla.

**Keywords:** *catla catla, laboe rohita, backcross, esterase.*

**GJSFR-G Classification :** FOR Code: 290101

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I. **Introduction**

Revolutions in agriculture and animal sciences were the boons for civilization. Running parallel to these achievements, fisheries also created a significant impact on the life style of common man throughout the globe. Aquaculture gained a status of industry from home stead activities in late nineties in Indian subcontinent. The broad arena of aquaculture encompasses captive fishery with tremendous potential in food production. Lannan et al. (1989) indicated the global demographic trend for urgent need of fisheries for human health irrespective of per capita animal fat consumption. As per Manna (1989), fishes serve the best source of animal proteins where people prefer fish to meat. The concept of harnessing animal proteins from aquatic organisms created fascination towards aquaculture, but it still remains a dream to bring much-awaited ‘blue revolution’ even though it has been described as a low-energy costing practice requiring less input for protein yield.

More than half of the population in developing countries get at least 40% of animal proteins, from fish (F.A.O., 2000) and India contributed around 3.6 million tons to global freshwater fish production in 2009. Indian aquaculture is highly promising and has grown over six times in the last four decades with freshwater aquaculture contributing over 95% of the total freshwater fisheries production. The two Indian Major Carps viz. *Catla catla* (Ham.) and *Labeo rohita* (Ham.) contributing lion share to aqua farming are the most imperative for freshwater aquaculture in Indian peninsula being widely adopted throughout the sub-continent due to their culture potential, availability and market demand. But it is always realized that, the larger head per unit body weight of catla is a major disadvantage (Basavaraju et al. 1995) so far as edible flesh content per unit body mass is concerned. Rohu is scattered naturally in various river systems of India (Jhingran and Pullin, 1985) and is one of the world’s principal aquaculture species in terms of production (Hulata, 2001) as per Islam and Alam (2004). Therefore, a good amalgamation of deep catla type body and narrow rohu type head is always a notion of considerable importance for aquaculture requiring apt hybridization. As the existing variations within a group of individuals provide ready opportunity towards preferred progenitors for selection, the idea of genetic evaluation on the line of varietal improvement by backcrossing is the core of the experimental design for the present study. A need was felt to improve the species/variety through introgression of some desirable gene(s) or quantitative trait(s) as hinted earlier by Sinha and Khan (1989) as well as Padhi and Mandal (1996) through backcrossing. Backcrossing is a well-known and long established breeding scheme where a characteristic is introgressed from a donor parent into the genomic background of a recurrent parent. Sinha and Khan (1989) discussed under inter-generic hybridization that, $F_1$ of catla x rohu may be allowed inter se breeding to develop $F_2$ progenies or backcrossed with parental species to establish new characters. Selection in backcross programmes is used to either improve the genetic value of plant and animal populations or fine map quantitative trait loci. Both cases are helpful in our understanding of the genetic bases of quantitative traits variation (Hospital, 2005). Backcrossing isolates a gene or chromosomal region in a different genetic background (the genetic background of the recurrent parent), it helps to dissect the genetic architecture of quantitative traits.

The relevance of biochemical tool for revealing the usefulness of the genetic architecture in an organism including fishes can be well understood since last two and half decades of research in various part of the globe from Hames and Rickwood (1990), Oliver et
al., 1991), Van der Bank et al., 1992), Buth, 1993, Sarangi and Mandal, 1996, Skibinski and Ward, 1998, Arai and Mukaino, 1998, Sarangi et al., 1999, Sarangi and Mandal, 2000, and Sarangi et al., 2002. As per Holmes and Whitt, 1970, there were a large number of loci responsible for a complex group of enzymes like esterases in vertebrates. In the context of genetic evaluation of the backcross progenies, it was realized that, esterase marker as a potential biochemical genetic tool must be employed to gain some view in to the hereditary trend. Biochemical genetics involving isozyme/allozyme analysis as an important genetic marker was extensively used to characterize plant genetic resources (Tanskley and Orton, 1983 and Heun et al., 1994). Tanskley and Orton (1983) reviewed the application of esterase as markers in plants where as Weeden et al., 1984 discussed the importance of esterase as biochemical marker in bean yellow mosaic virus resistance. Stuber et al., 1987 used this technique to manipulate quantitatively determined characters as per Powell, 1992. Takada (2004) reported the usefulness of esterase as genetic markers to study the composition and its dynamics in M. persica populations.

Reddy and Laxmipathy (1990) reported 30 esterase bands in various tissues of rohu with wide variations in partition coefficient (RI) values between individuals from adjacent collection sites of the same river system indicating a high degree of genetic differences. Mucous of rohu showed three esterase bands in two regions (Padhi and Khuda-Buksh, 1990). As per Chatterjee (1994) esterases are the most extensively surveyed isozymes in animal models. Among the Indian Major Carps and other cyprinid species in South Asia, many species-specific markers have been detected, using esterase (Gopalakrishnan, 1997).

Keeping this in view, the present study was undertaken to perform breeding of different backcross generations of catla and rohu with an aim to develop a new variety/strain with desirable traits. Biochemical analysis through esterase profiling was done for genetic evaluation of the developed progenies. As no such study in backcross generations of catla and rohu with respect to their parental generations are available, it may prove useful in future related to their genetics.

II. MATERIALS AND METHODS

a) Production of backcross progenies

Backcross generations of catla and rohu were developed in Central Agricultural Research Institute (CARI), Port Blair, South Andaman through systematic breeding approach from the founder stocks of the parental generations developed from the seeds procured from the hatchery unit of Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar, Odisha during 1987 as a part of hatchery development programme in CARI (Tripathy et al., 2010). The mating design followed was mostly 1:1 brooders for experimental purpose where as it was 2:3 or 1:3 (male: female) for farm requirement to meet the demand of local farmers and entrepreneurs every year. The F1 hybrids were developed by crossing catla female and rohu male and were designated as C x R or C R. Subsequently, the F2 hybrids were produced from inter se breeding of F1 progenies (CR x CR), the first backcross generation or B1 was produced from F1 and catla (CR x C), where as B2 from F1 and rohu (CR x R). Hybridization of B1 and rohu resulted in B1R (CR x C) x R and BC1F2 were from inter se breeding of B1 (CR x C) x (CR x C). The breeds were maintained in separate ponds and pools without allowing any mix up. Side by side various tissue samples were collected and preserved for future use.

b) The specimen

A total of 15 individuals from each generation of carps like catla, rohu, F2, B1R and BC1F2 ranging in size from 30-40 mm were selected for the purpose. The skeletal muscles, heart, liver and kidneys were collected with utmost care at sub-normal temperature arranged in ice packed boxes to prevent degradation of tissues and stored immediately after collection under refrigeration for future use. In case of BC1F2 some whole specimens were selected randomly ranging from 7.0 to 8.0 mm at hatching stage and crushed.

c) Esterase profiling

Esterase profiling was done (EST-1*, EST-2*, EST-3*, EST-4*, EST-5*, EC: 3.1.1.) as per Sarangi and Mandal (1996) following Abersold et al., 1987. The followed experimental protocol was for vertical slab gel electrophoresis (5% native polyacrylamide gel) in discontinuous buffer system (Rechardson et al. 1986) using Bio-Rad made mini electrophoretic apparatus (Mini Protein-II, Catalogue no 165) at 4ºC with running voltage of 250 V for 2 hours. The staining recipe was of Shaw and Prasad (1970), using substrate as 1% α, β naphthyl acetate made of α-naphthyl acetate 1 g and β naphthyl acetate 1 g dissolved in Acetone 50 ml and H2O 50 ml. Oliver et al., 1991 reported that, naphthol acetates might not be suitable for exhibiting sites of low esterase activity as those substrates were hydrolyzed more slowly than naphthyl acetate. The stain was made of Fast Blue RR 100 mg dissolved in 10 ml of 0.5 M TRIS of pH 7.1 and 87 ml of 1% α, β naphthyl acetate and 3 ml of H2O. The gel was incubated at room temperature until blue band appeared and then the gel was washed and fixed.

The co dominant isozyme bands were assigned different codes like aa, AA, Aa, BB and bb for homozygous conditions and AB, Ab, aB, bB for heterozygous conditions of loci. The data based on isozyme polymorphism of esterase markers were analyzed and the genetic distance matrix was
constructed by POPGENE-32. The dendogram was constructed by employing the statistical software based on original formulae of Nei (1972, 1978) for similarity index and genetic distance correlation by Unweighted Paired Group Method with Arithmetic Averages (UPGMA). It calculated the genetic distances and identities based on the formulae of pairwise similarities (sAB) as per Lynch (1990) originally based on Nei (1972) and Nei (1978) for similarity index, using the data for sAB = 2 NAB/(NA+NB), where NAB is the number of common bands between individuals A and B, and NA and NB are the total number of bands possessed by individuals A and B. The mean pair-wise similarity S was computed as S = \sum sAB/n where it is the arithmetic mean of all S values. As per Lynch (1990), the variance of S was calculated as V(S) = 2S(1-S)(2-S)/N(4-S). N stands for average number of isozyme bands per individual.

III. Results

a) Esterase profile

The result of esterase profiling is presented in table 1, 2, 3 and Figure 1 as well as the photographic plates 1, 2 and 3 summarizing the information on its polymorphism for comparison giving the values of genetic distance correlation, probability of genetic distance correlation and genetic distance matrix of co-ancestry identity and Nei’s genetic distance respectively.

Correlation of genetic distance (Theta) based on the polymorphism in the band pattern of various loci for esterase (Table 1) for catla indicates highest distance correlation with rohu (0.57), which is considered obvious due to their difference at generic level. Catla is lowest correlated to BC1F2 then other generations. The distance of catla with F2, B1R and BC1F2 are 0.42, 0.51 and 0.38 respectively. When the distance of various generations with rohu is considered, F2 shows highest correlation with respect to genetic distance (0.60). Lowest observation is with BC1F2 (0.02). B1R generation is more distinctly correlated to catla (0.51) than rohu (0.03) and with BC1F2 (0.02) and F2 (0.12). The BC1F2 backcross generation shows more distance with catla (0.38) followed by F2 (0.05) and with rohu and B1R (0.02). The correlation for genetic distance between both the backcross generation i.e. B1R and BC1F2 is 0.21. The probability of genetic distance correlation (Theta P) for esterase for all the loci analyzed shows highest value (0.22).

The Table 2 presents the genetic distance matrix of co-ancestry identity for esterase based on the loci analyzed for polymorphism. That of catla based on genetic distance matrix is highest with rohu (0.50) and lowest with BC1F2 (0.25). The co-ancestry of catla and B1R is more (0.42) than that with F2 (0.31). Co-ancestry of rohu is highest with catla and lowest with B1R (0.02). Those of rohu with BC1F2 and F2 are 0.03 and 0.07 respectively. The B1R shows highest co-ancestry identity with catla (0.42) and lowest with rohu and BC1F2 (0.02). That with F2 is 0.03. The BC1F2 shows the co-ancestry identity in ascending order with F2 (0.002), B1R (0.022), rohu (0.03) and catla (0.25).

Table 3 presents the genetic distance values as per Nei (1972) calculated by the software POPGENE-32 based on the polymorphism for esterase loci in various generations of catla and rohu. Catla shows Nei’s distance of 0.21, 0.22, 0.29 and 0.42 with F2, BC1F2, B1R and rohu respectively in ascending order. Highest value of rohu is with catla followed by BC1F2 (0.05), F2 (0.03) and B1R (0.033). The F2 shows maximum distance with catla (0.21) followed by rohu (0.03), B1R (0.025) and BC1F2 (0.02). B1R shows maximum distance with catla (0.29) and minimum with F2 (0.025). This generation is at a distance of 0.03 with rohu and 0.02 with BC1F2. The BC1F2 generation shows maximum genetic distance with catla (0.22) followed by rohu (0.05), B1R (0.02) and F2 (0.02).

Based on the genetic distance matrix of esterase, the constructed genetic distance tree i.e. the dendogram in the figure 1 shows two groups of carps clustering rohu along with other carps generations and catla in a separate branch.

IV. Discussion

So far as breeding backcross generations of these Indian Major Carps is concerned, it is a new concept in terms of the products derived for genetics of both the carps. As both the species interbreed among each other, it is easy to induce them for inter-generic breeding but the hindrance of the technique is that both have long generation periods of 2-3 years, hence their care and maintenance is highly time consuming, tedious and labor intensive. Therefore backcrossing is not followed usually in catla or rohu. But it is a frequently followed conventional technique in various plant models with short generation period for different classical genetic analyses. This type of crossing is generally attempted in plants important to horticulture and in some lower group of animals like worms, insects, some fishes and mammals with short generation period. Although aquaculture organisms differ from agricultural mammals and birds in several important ways (e.g. higher fecundity and smaller post-embryonic size), the principles of selective breeding can also be applied to their genetic improvement. The success of breeding backcross progenies of catla and rohu and their genetic evaluation to find out the heredity may be attributed to nature itself, which accepted intra-generic hybridization resulting in viable hybrids which is due to their compatibilities at chromosomal level (Zhang and Reddy, 1990).

Historically, biochemical markers such as allozymes/isozymes are used for genetics where the
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product of a gene rather than the DNA sequence itself is examined (polymorphism is detected in the form of bands at different positions in electrophoresis for enzyme activity. Powell (1992) wrote on the importance of esterase as isozyme marker in plant genomics that, those were the most widely used protein markers in breeding programmes. Kobor et al. (2004) cited various examples of writing on the importance of esterase analysis in some legume symbiont bacteria.

Simonsen et al. (2004) reported allozyme studies in three species of Indian Major Carps viz.- Catla catla, Labeo rohita and Cirrhinus mrigala revealing two number of loci for esterase and many more in other isozymes/allozymes in different tissues i.e.- eye and skeletal muscles and observed high incidence of hybridization in hatchery stock. Allelic frequencies of those loci as reported by Simonsen et al. (2004) were 0.18, 0.82 in rohu and 1.0 in catla for EST-1 locus with two alleles, 0.63, 0.37 in catla and 0.77, 0.18, 0.05 in rohu for EST-2 locus with three alleles. The present study exhibits presence of four loci (EST-1*, EST-2*, EST-3*, EST-4*, EC: 3.1.1.) in contrast to Simonsen et al. (2004) for catla, rohu as well as all the backcross generations.

In the present study, the dendogram on esterase profile based on genetic distance matrix showed two distinct clusters of carps linked together where one with rohu and excluding catla keeping aside in a separate branch. This indicated strongly that, all the generations of carps are linked more closely to rohu then catla. This isozyme marker proved to be highly significant to reveal segregation pattern in various backcross generations of carps - rohu then catla. This isozyme marker proved to be highly significant to reveal segregation pattern in various backcross generations of carps - rohu then catla. This isozyme marker proved to be highly significant to reveal segregation pattern in various backcross generations of carps- rohu then catla. Maximum genetic distance with catla followed by rohu, BC 1F2, F2 and B1R. BC 1F2 showed maximum genetic distance with catla followed by rohu, BC 1F2, F2 and B1R.

V. Acknowledgements

The authors highly acknowledge the constant moral support and guidance provided during every step of this work to the then Director of Central Agricultural Research Institute (CARI), Port Blair, South Andaman allowing the facilities and materials to be used for the purpose.

References Références Referencias


**Table 1**: Genetic distance correlation (Theta) based on polymorphism of esterase

<table>
<thead>
<tr>
<th>Theta</th>
<th>Catla</th>
<th>Rohu</th>
<th>F₂</th>
<th>B₁,R</th>
<th>BC₁F₂</th>
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<tbody>
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<td>Catla</td>
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<tr>
<td>Rohu</td>
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<tr>
<td>F₂</td>
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<tr>
<td>B₁,R</td>
<td>0.51</td>
<td>0.03</td>
<td>0.12</td>
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<tr>
<td>BC₁F₂</td>
<td>0.38</td>
<td>0.02</td>
<td>0.05</td>
<td>0.02</td>
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</table>

*The values of F₂ are the means of three distinct types of F₂ progenies based on their morphotypes similar to catla, rohu and F₁, *** Minimum correlation of genetic distance (0.00),

**Table 2**: Genetic distance matrix of co-ancestry identity for esterase

<table>
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<th>B₁,R</th>
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<td>Catla</td>
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<td>BC₁F₂</td>
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<td>0.002</td>
<td>0.02</td>
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*The values of F₂ are the means of three distinct types of F₂ progenies based on their morphotypes similar to catla, rohu and F₁, *** Closest genetic distance (0.00)

**Table 3**: Genetic distance values as per Nei (1972)

<table>
<thead>
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<th>Theta</th>
<th>Catla</th>
<th>Rohu</th>
<th>F₂</th>
<th>B₁,R</th>
<th>BC₁F₂</th>
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<td>Catla</td>
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<tr>
<td>Rohu</td>
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<tr>
<td>F₂</td>
<td>0.21**</td>
<td>0.03**</td>
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<td>B₁,R</td>
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***The values of F₂ are the means of three distinct types of F₂ progenies based on their morphometry similar to catla, rohu and F₁, *** Closest genetic distance (0.00)