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Developing Backcross Generations of Indian Major Carps viz. *Catla catla* (Ham.) and *Labeo rohita* (Ham.) by Induce Breeding in Captive Conditions of Bay Islands

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Abstract: The present study was undertaken to develop different backcross generations of *Catla catla* (Ham.) and *Labeo rohita* (Ham.), i.e., B_1 (CRxC), B_2 (CRxR), B_1R (CRxC)x R and BC_1F_2 (CRxC)x(CRxC) in Central Agricultural Research Institute (CARI), Port Blair, South Andaman, India, using the technique of induce breeding through hypophysation. Three different inducing hormones viz-Pituitary Gland Extract (PGE), Ovaprim and Ovatide were employed as per their recommended dose. Full release of eggs followed by good hatching in different experimental sets depicts the success of breeding. It is the first application of the concept of backcross breeding in IMC like rohu and catla. All the hybrids and backcross generations developed were highly viable and fertile. The success of breeding the backcrosses may be attributed to nature itself which accepted inter-generic hybridization resulting in viable hybrids. The success of developing the backcross generations is significant for carp genetics with special reference to catla and rohu.

INTRODUCTION

Fresh water aquaculture in India is principally based on three Indian Major Carps (IMC) viz. *Labeo rohita* (Ham.), *Catla catla* (Ham.) and *Cirrhinus mrigala* (Ham.), popularly known as rohu, catla and mrigal, respectively. They are scattered naturally in various river systems of India, Pakistan, Burma and Bangladesh (Jhingran and Pullin, 1985). Manna (1989) reported them as excellent source of high quality, easily digestible animal proteins and these are among the world's principal aquaculture species in terms of production (Hulata, 2001).

As per estimation by FAO. (2000), more than half population in developing countries like India get 40% animal proteins from fish. Mostly, low-income food-deficit Asian countries, account for nearly 85% of the world's aquaculture production with growth rate of 9% per annum which was projected to contribute 41% (53.6 million tonnes) of the world's fish production by 2020 (Rana *et al.*, 2009). As per the recent report by Meher *et al.* (2014), contribution of these carps is 1.8 million tonnes annually, figuring more than 80% of the total national aquaculture production.

Larger head of catla is considered as a major disadvantage for freshwater aquaculture in terms of edible flesh content per unit body mass (Basavaraju *et al.*, 1995). A good amalgamation of deep body like catla and narrow head like rohu is always a notion of considerable importance for aquaculture requiring apt hybridization. Sinha and Khan (1989) as well as Padhi and Mandal (1996) urged for the need of backcrossing to inherit desirable gene (s) of considerable economic trait (s) for greater flesh content. Keeping this in view, the present study was undertaken to develop backcross generations of *Catla catla* (Ham.) and *Labeo rohita* (Ham.) in Central Agricultural Research Institute (CARI), Port Blair, South Andaman, India using the technique of induced breeding through hypophysation. It is significant for genetics of IMCs with special reference to catla and rohu.

MATERIALS AND METHODS

The hatchery was a portable, circular tank of dimension 3' diameter ×2' depth made of plastic pool (China Model) supplied with ample facilities of water circulation, aeration and showering through perforated pipes. The base stocks were developed from the seeds of

Catla catla (Ham.) and *Labeo rohita* (Ham.) in farm facilities of CARI, Port Blair. (Tripathy *et al*., 2010) procured from the hatchery unit of Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar, Odisha as a part of institutional hatchery development programme.

A total of 10 different rain fed freshwater ponds maintained under semi-intensive farm management practice in facilities of CARI at different locations of Port Blair were employed. Regular cleaning of those ponds and treating with lime from time to time was done. The pH of water and soil often remained below neutral (5.5-6.8) posing challenging task for culture of freshwater fishes in captive conditions of Bay islands in and a mans. Standard mating design protocol was followed involving a long and tedious process of breeding, hatchery management and maintaining the breeds in isolated ponds. Different backcross generations of IMC viz. -B_1, B_2, B_1R and BC_1F_2 were developed where the F_1 hybrids were product of catla female and rohu male designated as C×R or CR. Subsequently, the F_2 hybrids were developed from *inter se* breeding of F_1 progenies (CR×CR). The first Backcross generation (B_1) of catla and rohu was developed by selective hybridization of maternal F_1 hybrids and paternal catla (CR×C) whereas B_2 generation was developed from F_1 female and rohu male (CR×R). Subsequent generations were developed from maternal B_1 and paternal rohu resulting in B_1R i.e., (CR×C)×R and BC_1F_2 was developed by inter se breeding of B_1 i.e., (CR×C)×(CR×C). The carps were supplied with feed in two different forms ad libitum for nursery rearing as well as brood stock development. One was a commonly used powdered feed prepared from locally available ingredients such as rice bran, ground nut oil cake (1:1) with vitamin-mineral premix @ 3% of stocked fish biomass by weight in fixed places of the ponds once in a day in feeding bags during the morning hour. The second feed was supplied at the onset of every breeding season to selected brooders acclimatized in various ponds to make them ready to use. It was a cooked one in dough form comprising broken rice, pulses, molasses, edible oil, vitamin and mineral premix for a fixed period of 2-3 months only.

Usually, brooders were netted out from the ponds in the fore noon of the day for preconditioning in submerged closed breeding happas (2.0×1.0×1.0 m^3) followed by selection based on maturity status. Selection of healthy brooders was done taking utmost care with lowest/minimum level of disturbance. The selected brooders were free from bruises, injuries or pathogenic infestations. Matured males were found with roughness like sand particles on pectoral fins and oozing out milt by slight gentle pressure where as the matured females were with bulging, uniformly round belly and pinkish vent. The breeding environment was maintained to favourable temperature (27-29°C) with alkaline pH of water. In adverse situations, breeding conditions were simulated in the hatchery by circulation of filtered water, fanning/aeration and showering. The hormones were injected intra-peritoneal near the pectoral fin, below the dorsal fin or at the base of caudal fins of brooders with slight deviations from standard protocols (Chaudhuri and Alikunhi, 1957; Alikunhi and Chaudhuri, 1959; Chaudhuri, 1959, 1973). Spot decisions were taken regarding the doses and durations depending upon the maturity status of brooders and weather conditions. The second dose when required was administered at an interval of 3-4 h.

Recommended doses of hormone like Pituitary Gland Extract (PGE) and synthetic hormones under the trade names Ovaprim and Ovatide were employed for successful induce breeding. At the beginning of each breeding season, PGE was prepared fresh by crushing preserved pituitary glands of carps and catfishes procured from local market of Kolkata @ 40 mg of tissue/mL in a mixture of solution containing 1:3 distilled water and glycerine. Finally, it was centrifuged at 2000 rpm and filtered to keep in ready to use form. Ovaprim was a combination of salmon gonadotropin releasing hormone (s Gn RH-A) (D-Arg6 Pro9-Net) and domperidone (DOM) in propylene glycol @ 20 µg of gonadotropin releasing hormone (sGnRH) and 10 mg of domperidone per mL. It was manufactured by Syndel Laboratories Inc., Vancouver Canada and marketed by M/s Glaxo Laboratories India (Ltd.). Similarly, Ovatide was another synthetic hormone produced by same manufacturer and marketed in India by Hemmo Pharma, Mumbai. The ingredients in one ml of original stock were 10 µg of gonadotropin releasing hormone (sGnRH) and 20 mg of domperidone. The biological active ingredients of Ovaprim and ovatide are nearly the same but ovatide has low viscocity and low cost as compared to Ovaprim (Dhawan and Kaur, 2004).

RESULTS AND DISCUSSION

Figure 1-4 and the Table 1-4 depict the success of breeding and developing various backcross generations of catla and rohu. The carps spawned after 5-6 h of final administration of the inducing hormones usually in the early morning. Sometimes they released their milt spontaneously after attaining right breeding condition or required manual stripping (hypophysation) and the milts were mixed with eggs by gentle stirring by soft feathers. Various permutation and combinations for inducing by PGE is clear from Table 1 to achieve optimum success. It was injected @ 6.0 mg/kg body weight of catla to females as 1st dose and 12.0-14.0 mg/kg body weight as second dose. A single dose to catla male varied from 4.0-7.0 mg/kg body weight. No second dose of PGE was

Table 1: Doses of PGE used for induce breeding (mg/kg body weight)

	♀		♂			
Carps	1st	2nd	1st	2nd	Duration (h) in between doses	Duration (h) for egg release after 2nd dose
Catla catla	6.00	12.00-14.00	4.00-7.00	NA	5.00	5.00-6.00
Labeo rohita	5.50-6.00	12.00-14.00	NA	5.00-6.00	5.00-5.50	5.00-6.00
NA	5.50-6.00		NA	12.00-14.00	5.00-5.50	5.00-6.00
F_1 (C×R)	6.00	12.00-14.00	6.00	NA	4.00-5.00	4.00-5.00
B_1 (CR×C)	6.00-12.00	6.00-12.00	6.00	NA	5.00	5.00

NA: Not Applicable

Table 2: Doses of Ovaprim used for induce breeding (mL/kg body weight)

	♀		♂			
Carps	1st	2nd	1st	2nd	Duration (h) in between doses	Duration (h) for egg release
Catla catla	0.50	NA	0.15	NA	NA	5.00-6.00
Labeo rohita	0.40	NA	0.20	NA	NA	5.00-6.00
F_1 (C×R)	0.45	NA	0.20	NA	NA	5.00-6.00
B_1 (CR×C)	0.40	NA	NA	NA	NA	5.00-6.00

NA: Not Applicable

Table 3: Doses of Ovatide used for induce breeding (mL/kg body weight)

	♀		♂			
Carps	1st	2nd	1st	2nd	Duration (h) in between doses	Duration (h) for egg release
Catla catla	0.50	NA	0.20	NA	NA	5.00-6.00
Labeo rohita	NA	NA	0.20	NA	NA	5.00-6.00

NA: Not Applicable

Table 4: Detail of induce breeding of various carp generations

Developed carp generations	Inducing hormones	Egg released (L)	Mean egg released (L)	Dry weight of egg (kg)	Mean dry weight of egg (kg)	Spawn recovered (thousand)	Mean spawn (thousand) recovery
F_1 (C×R)	PGE-PGE	11.00	16.33	0.40	1.63	5.00	106.66
	PGE-PGE	20.00		2.40		180.00	
	PGE-OVT	18.00		2.10		135.00	
	Total	49.00		4.90		320.00	
F_2 (CR×CR)	OVP-OVP	5.00	19.0	0.20	1.66	1.00	230.50
	PGE-PGE	28.00		2.60		460.00**	
	PGE-OVP	24.00		2.20			
	Total	57.00		5.00		461.00	
B_1 (CR×C)	PGE-PGE	4.50	8.16	0.30	0.61	5.00	17.33
	PGE-PGE	8.00		0.60		12.00	
	PGE-PGE	12.00		0.95		35.00	
	Total	24.50		1.85		52.00	
B_2 (CR×R)	PGE-OVT	7.00	16.66	0.35	1.00	6.00	35.00
	PGE-PGE	21.00		1.30		46.00	
	PGE-PGE	22.00		1.35		53.00	
	Total	50.00		3.00		105.00	
B_1R (CR×C)×R	OVP-PGE	7.00	6.00	1.50	0.90	50.00	25.66
	PGE-PGE	5.00		0.20		13.00	
	OVP-PGE	6.00		1.00		14.00	
	Total	18.00		2.70		77.00	
BC_1F_2 (CR×C)× (CR×C)	PGE-PGE	10.00	9.33	1.20	1.26	180.00	150.00
	PGE-PGE	14.00		1.80		225.00	
	PGE-PGE	4.00		0.80		45.00	
	Total	28.00		3.80		450.00	

PGE: Pituitary Gland Extract, OVP: Ovaprim, OVT: Ovatide, ** Spawn was spoiled

required to induce catla, F_1 and B_1 male brooders where as the rohu males were induced at the time of second dose administration @ 5.5-6.0 or 12.0-14.0 mg.kg body weight. Catla, rohu and F_1 females required 1st dose of PGE @ 5.5-6.0 mg.kg body weight where as B_1 females required a higher dose (6.0-12.0 mg/kg). The second dose of PGE to all female carps comprised 12.0-14.0 mg.kg body weight except that in B_1 which was 6.0-12.0 mg/kg body weight. The average duration between the two doses comprised 4.0-5.5 h where as the average time interval of egg release after the last dose of administration was 4.0-6.0 h.

The synthetic hormone with trade name Ovaprim was applied as per standard recommendation of the

Fig. 1: Photographs of representative carps of different generations

Fig. 2: The parental generations of backcrosses

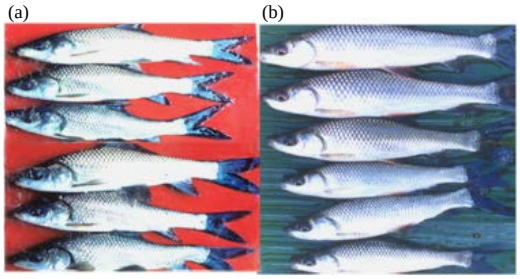

Fig. 3(a-b): Backcross generations of *Catla catla* and *Labeo rohita* (a) Fingerlings of BC_1 F_2 and (b) B_1 R progenies

manufacturing company (Table 2). No second dose was required to any of the brooder. Duration for release of egg after administration was 5.0-6.0 h. Females required 0.4-0.5 mL/kg where as males required 0.15-0.20 mL/kg body weight.

Similarly, Table 3 describes the application of the second synthetic hormone Ovatide for breeding purpose. It was applied only to catla and rohu in a single dose. Catla females were induced @ 0.5 mL/kg and males @ 0.20 mL/kg body weight. Only male rohu were induced by the hormone @ 0.20 mg/kg body weight. The spawning success (fecundity and fertilization rate) of *C. catla* as per Dhawan and Kaur (2004) is more with Ovaprim whereas the results were better with Ovatide in *L. rohita* and *C. mrigala.* Reddy and Mathur also reported higher success of ovatide in *L. rohita* and *C. mrigala* as compared to *C. catla*. Chauhans *et al.* (1999) reported the breeding success of *L. rohita* at par when induced to breed with Ovaprim and ovatide.

Table 4 presents the overall summary of success achieved in terms of egg release and spawn recovery for various developed carp generations. In a total set of 18 breeding experiments only a single case was a failure while developing the F_2 generation. In this experiment, while the female F_1 hybrids were induced by PGE and the male F_1 hybrids were induced by OVP. During this attempt 24 L of eggs was released successfully mounting to 2.2 kg dry weight but the spawn recovery failed due to spoilage of eggs resulting from some technical error during transfer of eggs or sudden drastic change in environmental condition caused by heavy, erratic rain fall or change in pH of the pond water. In rest other experiments, successful egg release and spawn recovery was achieved.

Development of B_1 backcross generation is depicted in three experiments using PGE as the only inducing hormone to the parental F_1 hybrid females and catla males. A total of 24.5 L of eggs was released with an average of 8.16 L per experiment mounting to a total dry weight of 1.85 kg (Mean 0.61 kg). These resulted in a total production of 52 thousand B_1 backcross spawn with an average of 17.33 thousand spawn per experiment.

B_2 backcrosses (CRxR) were developed from F_1 hybrid female and rohu male in three experimental attempts as depicted in Table 4. A total of 105 thousand spawn were successfully recovered @ 35.0 thousand (mean) per experiments. Only in a single experiment, the parental rohu male was induced with OVT where as all other brooders like F_1 hybrid females and rohu males were induced by PGE. The mean egg release per experiment was 16.66 L mounting to mean dry weight of 1.0 kg.

In three different attempts to develop B_1R backcross generation (CRxC)xR, the synthetic hormone OVP was employed twice to induce maternal B_1 backcross brooders and all others were induced by PGE recovering a total of 77 thousand spawn. Similarly, a total of 450 thousand spawn of BC_1F_2 backcross generation, i.e., (CRxC)x(CRxC) were recovered from three different experiments involving B_1 backcross brooders only. All of them were induced by PGE only.

Lush (1945) laid the initial meaning for the word breeding as “the mean available for improving the heredity of farm animals”. It may be seen as “optimal exploitation” of the species “biological variations” under given constraints of reproductive capacity using appropriate breeding value estimation tools. Contribution of a particular locus or closely placed group of loci to the polygenic variations in terms of genetics of breeding turned out to be large due to result of earlier selection through systematic breeding approach (Mather, 1953). Ryman and Stahl (1980) concluded the fundamental

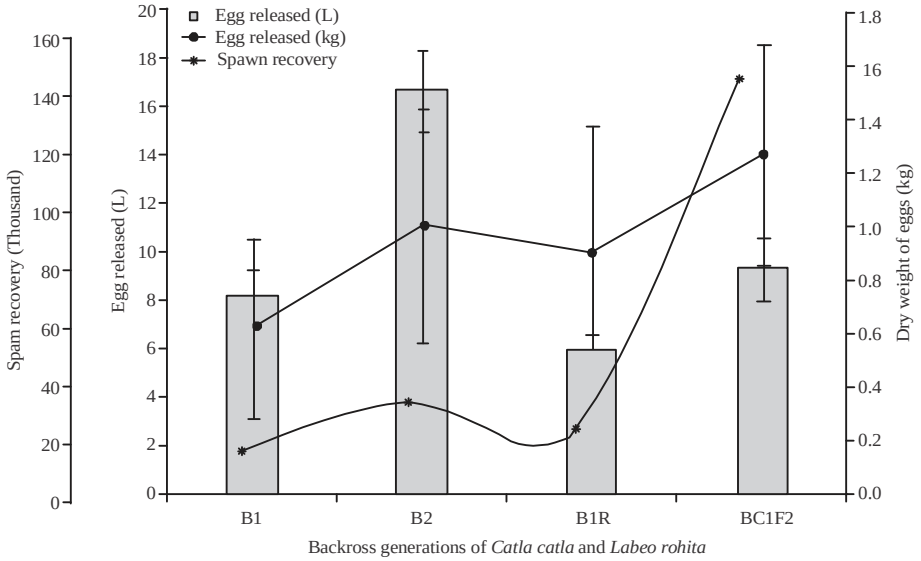

Fig. 4: Release of eggs in litre with corresponding dry weight in kg and the spawn recovery in thousand (Mean±SEM)

importance of breeding programme to prevent inadvertent damage to the genetic pool through loss of genetically determined population characteristics. As per Gjedrem (1985, 1992) and Pickering (1993), any breeding scheme/plan are to be traditionally based on some fundamental characteristics of concerned species i.e., growth rate and reproductive performance. The challenge is to determine exactly what each gene does in terms of the development and physiological functioning of the organism (Murphy, 2002). The study of the influence of gene expression in performance traits like growth rate, feed conversion efficiency, body conformation, disease resistance and sex determination is an opportunity to meet the demands of fish production while ensuring profitability (Liu, 2007). In this context, 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 (Hospital, 2005). Selection in backcross programmes is used to either improve the genetic value of plant and animal populations or fine map quantitative trait loci. It is also useful to dissect the genetic architecture of quantitative traits because it isolates a gene or chromosomal region in a different genetic background of the recurrent parent.

With an intention to improve the genetic architecture of IMC, their backcross generations were developed in CARI, Port Blair, to establish some of the desired morphometric characters such as small and narrow head of rohu as well as deep, broad body of catla. The experiments demonstrated successful breeding of various backcross generations by utilization of three inducing hormones viz. PGE, Ovaprim and Ovatide. However, development of backcross progenies in IMC is not a well adopted practice as they have long generation cycle of 3 years approximately. But similar attempts were made in some other carps and non-carps earlier (Behrends *et al.*, 1988; Anderson and Collins, 1995; Galbreath and Thorgaard, 1995) for various purposes of aquaculture in general as well as to understand their genetics.

CONCLUSION

Full release of eggs followed by good hatching in different experimental sets depict the success of breeding where only a single case during the production of F_2 resulted in spoilage of eggs. The breeding experiments with successful utilization of all the three inducing hormones in various permutation combinations are relevant to this study, though there were many chances of risk factors. Use of the synthetic hormone, i.e., Ovatide in particular was doubtful for the success though the manufacturing company recommended its use strongly. However, no such trial with Ovatide was made earlier in Bay island conditions.

In some previous attempts, hybrids between Atlantic salmon (*Salmo salar*, At) and brown trout (*Salmo trutta*, Bn) were highly viable and expected to be functionally sterile due to major inter-specific karyotypic differences (Galbreath and Thorgaard, 1995). In contrast to this in the present study, inter-generic differences of catla and rohu has not produced similar results but all the hybrids and backcross generations developed were highly viable and fertile which might be due to chromosomal compatibility with same diploidy. It is the first application of the concept of backcross breeding in IMC like rohu and catla.

The success of breeding the backcrosses may be attributed to nature itself which accepted inter-generic hybridization resulting in viable hybrids.

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