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Induce breeding to develop backcross generations of *Catla catla* (Ham.) and *Labeo rohita* (Ham.)

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Abstract
The 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, by induce breeding through hypophysation. Three different hormones viz- Pituitary Gland Extract (PGE), Ovaprim and Ovatide were employed as per recommendation. Full release of eggs followed by good hatching in different experimental sets depicted 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.

Keywords: Back cross, Carp genetics, Hypophysation, Indian major carps, Pituitary gland extract, Synthetic hormones.

1. 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 Pulin, 1985) [1]. Manna 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 (Manna, 1989, Hulata, 2001) [2, 3].

As per estimation of F.A.O. more than half population in developing countries like India get 40% animal proteins from fish (F.A.O., 2000) [4]. 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 (Krishen *et al.,* 2009) [5]. Contribution of these carps is 1.8 million tonnes annually, figuring more than 80% of the total national aquaculture production (Meher *et al.,* 2014) [6].

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) [7]. 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. The need of backcrossing to inherit desirable gene (s) of considerable economic trait (s) for greater flesh content was realized (Sinha and Khan, 1989, Padhi and Mandal, 1996) [8, 9]. 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 IMC with special reference to catla and rohu**.**

Materials and methods
2.1. *Hatchery*
The hatchery was a portable, circular tank of dimension 3' diameter x 2' depth made of plastic pool (China Model) supplied with ample facilities of water circulation, aeration and showering through perforated pipes.

2.2. *The base stock populations*
The base stocks were developed from the seeds of *Catla catla* (Ham.) and *Labeo rohita* (Ham.) in farm facilities of CARI, Port Blair procured from the hatchery unit of Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar, Odisha as a part of institutional hatchery development programme (Tripathy *et al.,* 2010) [10].

The ponds and management practices
A total of 10 different rain fed freshwater ponds were maintained under semi-intensive farm management practice in facilities of CARI at different locations of Port Blair, South Andaman, India. The pH of water and soil often remained below neutral (5.5 to 6.8) posing challenging task for culture of freshwater fishes in captive conditions of Bay islands in Andamans. It required regular cleaning of ponds and treating with lime from time to time.

Mating design and backcross generations of catla and rohu
Protocol of standard mating design 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).

Feed and feeding practice

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 and 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.

Pre breeding care

Usually, brooders were netted out from the ponds in fore noon for preconditioning in submerged closed breeding happas ($2.0\times1.0\times1.0$ 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 (27-29ºC) 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 inducing hormones

Recommended doses of hormones like pituitary gland extract (PGE) and synthetic hormones under the trade names Ovaprim and Ovatide were employed for successful induce breeding through hypophysation. 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; Nandeesha *et al.,* 1990) [11-15]. Usually, the second dose when required was administered at an interval of 3-4 h.

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 r. p. m. and filtered to keep in ready to use. The biological active ingredients of synthetic hormones like ovaprim and ovatide are nearly the same but ovatide has low viscocity and low cost as compared to ovaprim (Dhawan and Kaur, 2004) [16].

Ovaprim was a combination of salmon gonadotropin releasing hormone (s Gn RH-A) (D-Arg6 Pro9-Net) and domperidone (DOM) in propylene glycol @ 20µg 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.). 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 10µg of gonadotropin releasing hormone (sGnRH) and 20 mg of domperidone.

Doses of hormones applied

Various permutation and combinations for inducing by PGE is clear from Table 1 to achieve optimum success. It was injected @ 6.0mg/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 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 to14.0 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 to 6.0 h.

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

Sl. No	Carps	♀		♂		Duration (h) in between doses	Duration (h) For egg release after 2nd dose
		1st	2nd	1st	2nd		
1	*Catla catla*	6.00	12.00-14.00	4.00-7.00	NA	5.00	5.00-6.00
2	*Labeo rohita*	5.50-6.00	12.00-14.00	NA NA	5.50-6.00 12.00-14.00	5.00-5.50 5.00-5.50	5.00-6.00 5.00-6.00
3	F_1 (C × R)	6.00	12.00-14.00	6.00	NA	4.00-5.00	4.00-5.00
4	B_1 (CR × C)	6.00-12.00	6.00-12.00	6.00	NA	5.00	5.00

NA: Not applicable

Ovaprim was applied as per standard recommendation of the 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 to 0.20 ml/kg body weight.

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

Sl. No	Carps	♀		♂		Duration (h) in between doses	Duration (h) for egg release
		1st	2nd	1st	2nd		
1	*Catla catla*	0.50	NA	0.15	NA	NA	5.00-6.00
2	*Labeo rohita*	0.40	NA	0.20	NA	NA	5.00-6.00
3	F_1 (C × R)	0.45	NA	0.20	NA	NA	5.00-6.00
4	B_1 (CR × C)	0.40	NA	NA	NA	NA	5.00-6.00

NA: Not applicable

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 @ 0.5ml/kg and males @ 0.20 ml/kg body weight. Only male rohu were induced by the hormone @ 0.20 mg/kg body weight.

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

Sl. No	Carps	♀		♂		Duration (h) in between doses	Duration (h) For egg release
		1st	2nd	1st	2nd		
1	*Catla catla*	0.50	NA	0.20	NA	NA	5.00-6.00
2	*Labeo rohita*	NA	NA	0.20	NA	NA	5.00-6.00

NA: Not applicable

Results

Figures 1-4 and the Table 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.

Table 4: Detail of induce breeding of various carp generations

Developed carp generations	Inducing hormones ♀ - ♂	Egg released (L)	Mean egg released (L)	Dry wt. of egg (kg)	Mean dry wt. of egg (kg)	Spawn recovered (thousand)	Mean spawn recovery (thousand)
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

The overall summary of success achieved in terms of egg release and spawn recovery for various developed carp generations is presented in Table 4. In a total set of 18 breeding experiments only a single case was a failure while developing the F_2 generation. In this experiment, 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 because of 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.

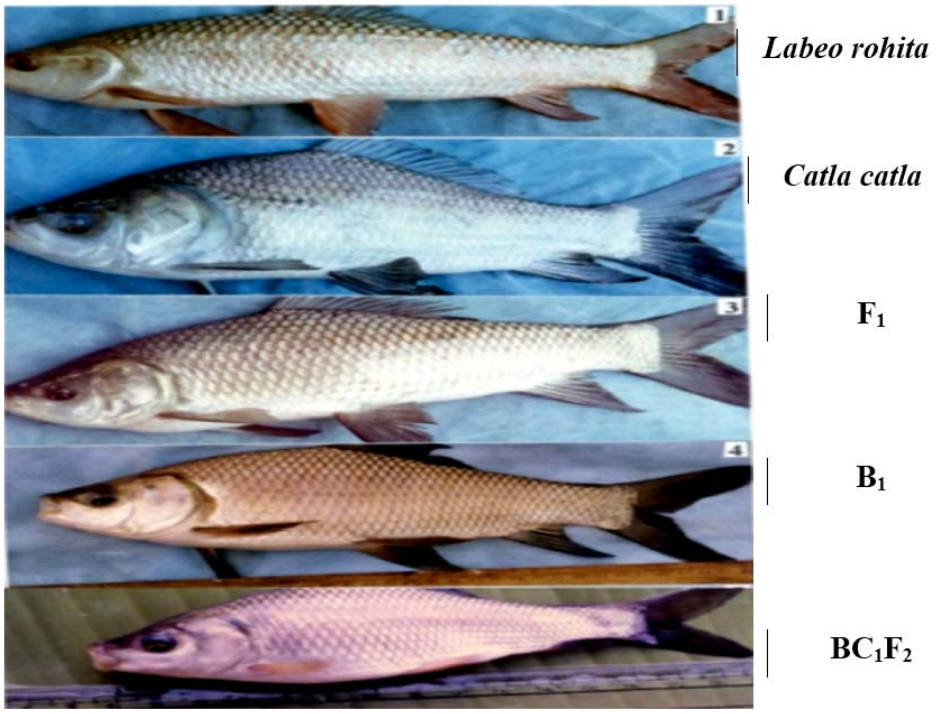

Fig 1: Photographs of representative carps of different generations

Fig 2: The parental generations of backcrosses

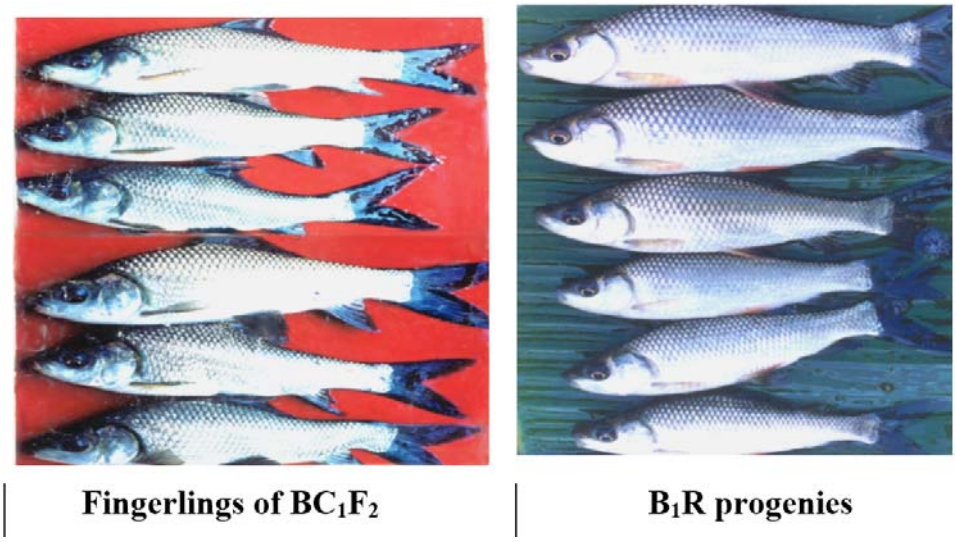

Fig 3: Backcross generations of *Catla catla* and *Labeo rohita*

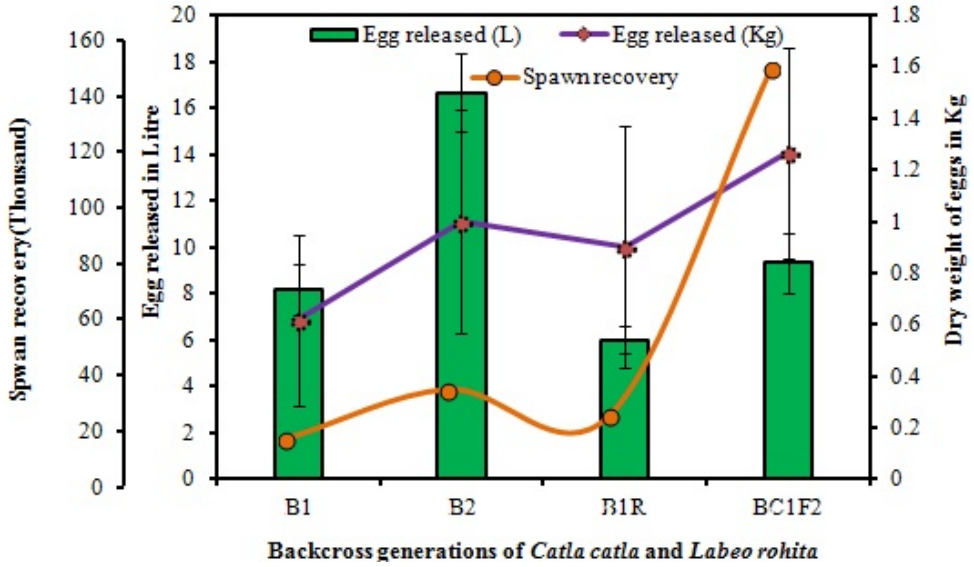

Fig 4: Release of eggs in litre with corresponding dry weight in Kg and the spawn recovery in thousand (Mean ± SEM)

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 (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.

Discussion

Lush laid the initial meaning for the word breeding as "the mean available for improving the heredity of farm animals" (Lush, 1945) [17]. 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) [18]. The fundamental importance of breeding programme is to prevent inadvertent damage to the genetic pool through loss of genetically determined population characteristics (Ryman and Stahl, 1980) [19]. Any breeding scheme/plan are to be traditionally based on some fundamental characteristics of concerned species i.e.- growth rate and reproductive performance (Gjedrem, 1985, 1992; Pickering, 1993) [20-22]. The challenge is to determine exactly what each gene does in terms of the development and physiological functioning of the organism (Murphy, 2002) [23]. 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) [24].

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) [25]. 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. The spawning success (fecundity and fertilization rate) of *C. catla* as reported earlier is more with ovaprim, whereas, the results were better with Ovatide in *L. rohita* and *C. mrigala* [16]. Reddy and Mathur (2000) also reported higher success of ovatide in *L. rohita* and *C. mrigala* as compared to *C. catla* (Reddy and Mathur, 2000) [26]. Chauhan *et al.* reported the breeding success of *L. rohita* at par when induced to breed with ovaprim and ovatide] (Chauhan *et al.*, 1999) [27]. However, in the present study, PGE was found to be most useful for induce breeding.

Conclusion

Development of backcross progenies of IMC is not a well adopted practice as they have long generation cycle of approximately three years. But similar attempts were made in some other carps and non-carps earlier (Anderson and Collin, 1995; Galbreath *et al.,* 1995) [28-29]. for various purposes of aquaculture in general; as well as, to understand their genetics. In the present study, 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. 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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