Bangladesh J. Fish. Res., 1(2) : 01-07 July 1997
Bangladesh Fisheries Research Institute

Induction of mitotic and meiotic gynogenesis and production of genetic clones in rohu, *Labeo rohita* Ham.

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Abstract

Studies were undertaken to produce genetic clones derived from all homozygous mitotic gynogenetic individuals in rohu, *Labeo rohita* Ham. In view of this, attempts were made to interfere with the normal functioning of the spindle apparatus during the first mitotic cell division of developing eggs using heat shocks, there by leading to the induction of mitotic gynogenetic diploids in the F_1 generation. Afterwards, viable mitotic gynogenetic alevins were reared and a selected mature female fish was used to obtain ovulated eggs which were fertilized later with UV-irradiated milt. Milt was diluted with Cortland's solution and the sperm concentration was maintained at 10^8/ml. The UV- irradiation was carried out for 2 minutes at the intensity of 200 to 250 μW/cm^2 at 28± 1^{0}c. The optimal heat shock of 40^{0}c for 2 minutes applied at 25 to 30 minutes a.f. was used to induce mitotic gynogenesis in fisrt (F1) generation and at 3 to 5 minutes a.f. to induce meiotic gynogenesis in the second (F2) generation. The results obtained are presented and the light they shed on the timing of the mitotic and meiotic cell division in this species is discussed.

Key words : *L. rohita,* Gynogenesis, Genetic clones

Introduction

Highly inbred strains of fish are advantageous for commercial and research purposes and have been identified as having great potential to the development of aquaculture (Komen *et al.* 1991, Hussain *et al.* 1993). Gynogenesis is useful for rapid improvement of genetic characters, producing inbred (clonal) lines compared with the traditional methods of sib-mating for upto 10 to 20 generations (Purdom and Lincoln 1973). Clonal lines are supposed to be very valuable products for improvement of fish stocks (Han *et al.* 1991). In view of

this, meiotic gynogens have been produced in many species of fish but are not completely homozygous due to recombination between non-sister chromatids (Purdom 1959, Hussain *et al.* 1994). This occurrence is undoubtedly absent in case of the production of mitotic gynogens induced by the suppression of first mitotic cleavage and thereby the resulting progeny are homozygous at every gene locus. Therefore, it is very advantageous for producing clonal lines in the subsequent generation(s) with unique gene combination Although earlier reports are available on meiotic gynogenesis in fish, the production of mitotic gynogens was first successfully initiated by Streisinger *et al.* (1981) and the technique has since been applied to a number of fish. Despite having produced mitotic gynogens, until now clones have only been successfully produced in zebra fish (Streisinger *et al.* 1981), medaka (Naruse *et al.* 1985), common carp (Komen *et al.* 1991), ayu (Han *et al.* 1991) and nile tilapia (Hussain 1992). Successful diploid gynogenesis, both mitotic and meiotic using methods of UV-irradiation of sperm and various physical and chemical shock treatments have been reported by many authors (for reviews, see Komen *et al.* 1991 and Hussain 1994). But until recently, no such report is available on the induction of mitotic gynogenesis and subsequent production of clones in Asian carps. Therefore, the present work was undertaken and the trials were aimed at the production of mitotic gynogens as a first step and secondly meiotic gynogens for the development of clonal lines in *Labeo rohita* Ham.

Materials and methods

Origin of broodstock and induced breeding

The gonadal materials, eggs and sperm, used in this study were obtained from different mature broods of *L. rohita* maintained under hatchery programmes of the Freshwater Station, Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh. The broods were induced with carp pituitary extracts through intramuscular injections (twice, six hourly) and after 11 to 12 hours the milt and eggs were collected by stripping.

UV-irradiation of milt

The milt was kept under refrigerated condition at 4^{0}c for a few minutes. The milt was then diluted at 1 :100 to 1:200 times with refrigerated physiological saline solution, Cortland's, solution with pH 7.2 to 7.5 (Wolf 1963) and samples from this were checked for motility of sperm. Then the concentration of sperm from samples were determined and maintained at a concentration of about 10^{8} ml^{-1} by further dilution. The diluted milt was spread out on to a plastic petridish and a fine film was made with a thickness of about 0.5-1.0 mm. This sperm solution was exposed to ultraviolet (UV) irradiation under a short wave UV-lamp (Model UVBGL-58; Multiband-254/366 NM). The intensity of UV-irradiation was

optimized at 200-250 μW/cm^2 applied for 2 minutes at 28±1^{0}c and the irradiated sperm was kept in a refrigerator controled at 4^{0}c. The irradiated sperm was then used to inseminate the eggs. Based on the preliminary experiment these parameters were fixed for subsequent trails.

Induction of gynogenesis (both mitotic and meiotic)

All the treatment batches of eggs were fertilized with UV-irradiated milt except the control eggs which were fertilized with untreated, diluted milt. The recently stripped eggs fertilized with UV-irradiated sperm were poured into an incubator jar of 6 litre capacity with a water flow rate at 1 litre/minute at a temperature of 26±1^{0}c for normal development. Heat shocks designed to interfere with the first mitotic cell division of these eggs were initiated from 10 minutes up to 50 minutes a.f. to determine the window at which treatment can properly be applied. The experimental parameters used to interfere with the first mitosis were at a late heat shock of 40^{0}c for 2 minutes, applied at 20 to 40 minutes after fertilization with 2.5 minutes intervals. These parameters were also used as optimal for the retention of second polar body in producing meiotic gynogens by early shocks, 3 to 5 minutes a.f.

Egg incubation and karyotyping

The temperature for incubation of eggs was always maintained at 26±1^{0}c. The rates of fertilization, hatching success of eggs and survival of spawn were recorded. The hatching of normal larvae was considered as the primary criterion for estimating the success of induced diploidization. The karyotypes of samples from treated and untreated groups were determined by the techniques as described by Hussain and Mcandrew (1994).

Production of genetic clones

The survivors of two batches of the induced mitotic gynogens attained an average weight of 1.8 and 1.9 kg in their second year of life and they were all female. Among them, only a single mature female was selected and this brood was used in the production of induced meiotic gynogens. The protocol for induction of meiotic gynogenesis is described above.

Results and discussion

Table 1 presents the rates of fertilization, hatching and survivals of different treated and control batches obtained in the production of mitotic gynogens in the first (F_1) and Table 2 for clones through meiotic gynogens in the second (F_2) generation, respectively. No survival was observed in non-shocked groups using UV-irradiated milt (Haploid gynogens) in which the embryos were found to be deformed and abnormal in structure, the karyological investigation reveals them

to be all haploid (n=25) whereas the karyotypes were found to be diploid (2n=50) in both meiotic and mitotic gynogens. Heat shocks of 40^{0}c for 2 minutes, which were optimal for the production of meiotic gynogenetics when applied at 3 to 5 minutes a.f. were also found to induce mitotic gynogenetics when applied between 25 to 30 minutes a.f. The event of first mitotic cell division was observed from 25 to 30 minutes and continued up to 35 tp 40 minutes a.f. at 26±1^{0}c. These results would support that the heat shock application was properly applied between 25 to 30 minutes a.f. for the production of mitotic gynogens. This result is very similar to that of Nagy (1987) and Komen *et al.* (1991) for common carp, *Cyprinus carpio* where they induced mitotic gynogens by heat shocks at 40^{0}c for 2 minutes applied at a window of 28 to 30 minutes a.f. The persent results are also consistent of with those of Mair *et al.* (1987) and Hussain *et al.* (1993), where they found optimal heat shock parameters for the production of mitotic gynogens in tilapia, *Oreochromis niloticus L.* to be at 41^{0}c for 3.5 minutes applied at the window of 28 to 35 minutes and /or 27.5 to 30 minutes a.f., respectively. In this study, the rates of fertilization, hatching and survivals up to yolk sac resorption stage were found to be comparatively much lower in both mitotic gynogens in the first and meiotic gynogens (clones) in the second generation than those of normal control groups. These low survivals of mitotic and meiotic gynogens might be due to their extreme homozygosity. Komen *et al.* (1991) found mitotic gynogens of common carp *(C. carpio)* being survived at 5 to 15%, whereas our results are somewhat lower at 2.5 to 4.8%. Survival rates of inbred individuals in fish may be species - specific.

Table1. Observation on the production of mitotic gynogenetics in rohu, *L. rohita*

Trial No.	Parameters	Normal Control (%)	Haploid Control (%)	Mitotic Gynogens (%)	Mitotic Gynogens (%)
	Fertilization	85	85	87	80
1	Hatching	46	02	05	07
	Viability at one week age	37	0	3.4	4.8
	Fertilization	100	97	97	90
2	Hatching	92	0.9	2.5	3.7
	Viability at one week age	35	0	1.67	2.5

In this study, late heat shocks at either side of 25 to 30 minutes produced no survival : most of the treated eggs and embryos died befor hatching and the rest deformed embryos having haploid syndrome. This would strongly suggests that the survivors (diploids) of treated baches with in the window of 25 to 30

minutes a.f. were viable mitotic gynogens. In other trials, the same heat shocks between 25 to 30 minutes a.f. were failed in producing mitotic gynogens. However, the undeveloped and less developed (comparatively slow moving) embryos produced from batches treated with this window for both successful and unsuccessful cases were karyologically revealed to be haploids and / or haploid/diploid mosaics. Hussain *et al.* (1993) and Hussain (1995) observed high frequencies of haploid/diploid mosaics on either side of optimal window of heat shocks. He also opined that the heat shocks at the extremes of the effective window and or sub-optimal shocks could result in the production of haploid /diploid mosaics. Our data would suggest that the protocol for the induction of mitotic and meiotic gynogenesis in *L. rohita* using heat shock parameters need to be critically optimized as the developmental asynchrony within a single batch of eggs results in haploids, mosaics and diploids apperaing together. Our investigation is in furture progress and we plan to establish different clonal lines in the near future which can probably be used in the production of hybrid vigour in this species.

Table 2. Observation on the production of F_2 'Clone' through meiotic gynogenesis using F1 brood from mitotic gynogens of rohu, *L. rohita*

Trial No	Parameters	Normal Control	Haploid Control : UV=200 μW cm^{-2} (	Haploid Control : UV=250 μW cm^{-2}	Mitotic gynogens I.e., clone
		(%)	%)	(%)	
1	Fertilization	98	76	-	68
	Hatching	92	0	-	5
	Survival at one week age	84	0	-	66
2	Fertilization	87	62	-	64
	Hatching	82	2	-	2
	Survival at one week age	84	89	-	66
3	Fertilization	98	-	72	74
	Hatching	92	-	0	12
	Survival at one week age	84	-	0	66
4	Fertilization	87	-	67	62
	Hatching	82	-	0	2
	Survival at one week age	84	-	0	66

Acknowledgements

The authors would thank Dr. D.J. Penman of Aquaculture Institute, University of Stirling, Scotland for his valuable suggestions and critically correcting draft manuscirpts.

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