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Chromosomal Studies on *Ompok pabda* (Hamilton)

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Abstract: Colchicine(0.05%) treated tissues of day-old larvae of ***Ompok pabda*** were observed under a research microscope after hydrolysis, mordanting and staining with 10% HCl, 2% aqueous solution of iron alum and 0.5% haematoxylin for 10 minutes, 6 minutes and 10 minutes respectively. Photomicrographs were taken from critically selected plates. From the photomicrographic enlarged prints chromosome number 2n=42 was counted, of which only 18 could be measured which consisted of 10 metacentric, 7 submetacentric and 1 subtelocentric. Length of the measured chromosomes varied from 1.1 to 3.9 μ.

Key words: Chromosome, ***Ompok pabda***, Colchicine

Introduction

The catfish ***O. pabda*** (Hamilton) locally known as pabda or pabo or madhu pabda is an indigenous freshwater small fish belonging to the family Siluridae of the order Siluriformes (Siddiqua ***et al.***, 2000). Owing to its delicious taste, pabda is a very favourite food fish of the people of Bangladesh. It has a high market value. Pabda is commonly found in natural waterbodies i.e. haors, baors, rivers, beels, and floodplains of Bangladesh. It is also found in India, Pakistan, Afganistan and Burma (Talwar and Jhingran, 1991).

Ompok pabda is a very popular food fish. Several unique features make it a perfect species for aquaculture i.e., it can be artificially bred in confined condition, it thrives well in small ponds, being omnivorous in nature its feeding is easy, it has a rather long breeding season from May to September and within one year become fully matured for breeding. Inspite of all these advantages, the population of ***O. pabda*** has declined drastically from the open waters. Already it has been declared as an endangered species. Very little attempt has been made in Bangladesh to promote its breeding and culture to protect it from extinction.

Today genetics has been a key area of research in protecting this fish. Though ***O. pabda*** is considered to be an endangered species and is genetically vulnerable, practically no attempt has yet been made to determine and improve the genetic status of it. So studies on chromosomal constitution and other genetic research is urgently needed enabling to undertake effective steps for the conservation of the species. The present research work was carried out to study the general cytological conditions of ***O. pabda*** and attempt to measure the metaphase chromosomes.

Materials and Methods

The research programme reported here was carried out during the period from July 2001 to October 2002 in the laboratories of the Department of Fisheries Biology & Genetics and Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh. The experimental procedures are described below:

Collection of sample

One-day-old ***O. pabda*** larvae were used for cytological studies. Healthy and vigorously growing larvae were sampled and allowed to swim in 0.05% colchicine solution for four hours at room temperature (28-29^{0}C) in appropriate sized petridishes. The larvae were then transferred into glass petridishes containing 0.7% NaCl solution. The head and yolksac of the larvae were then removed using fine forceps and needle while in the same solution for 15 minutes. The remaining body and tail tissues were then transferred to another petridish containing distilled water (hypotonic solution). After 10-15 minutes the tissues were transferred in a vial containing freshly prepared fixative (acetic acid: ethyl alcohol, 1:3). After a change of fixative the tissues were left in the fixative for slide preparation.

Hydrolysis of the sample

Hydrolysis of the fixed materials was done in 10% HCl solution to soften the tissue. HCl (10%) was kept in an oven at 60^{0}C for 1 hour in a tightly stoppered vial, prior to actual hydrolysis of the tissues. Fixed tissues were then kept in this preheated HCl solution in the oven for 10 minutes. After that the vial was taken out and the contents were kept in a watch glass for quick cooling. Then the tissues were washed thoroughly in running water.

Mordanting of the sample

The samples were then treated with 2% aqueous solution of iron alum (Ferric ammonium sulphate) for 6 minutes for mordanting.

Staining of the chromosomes

Iron alum treated tissues were then washed thoroughly and stained in 0.5% haematoxylin for 10 minutes. The stained tissues were then washed several times until colouring of water stopped. The stained samples were kept in water. Slides were prepared within 2-3 hours of staining.

Slide preparation and microscopic observations

A small portion of the stained tissue was placed on a clean glass slide in a drop of 1% aceto carmine solution. A clean cover glass was placed on the material avoiding any air bubble under the cover glass. The tissue was smeared by holding the cover glass with a finger on a corner and gentle tapping on the cover glass with the back end of the needle. Gentle warming of the slide over a spirit lamp and further tapping on the smear were repeated until the smear spread making a single layer thick without much overlapping in the cells, as well as avoiding much breakage of

the cells to enable clear observation of the cells under the microscope. Mitotic cells were observed using an Olympus research microscope.

Photomicrography and measurement of chromosomes

Photomicrography of the selected plates was done from temporary slides using an Olympus research microscope model BX 40, under 100X Plan Oil immersion objective. Chromosome counting and measurements of some selected chromosomes were done from photomicrographic prints. Such measurements were converted to micron (μ) based on the magnification of the final prints. Arm ratios of the chromosomes were calculated by dividing the length of the long arm by that of the short arm (L/S). The conventions proposed by Levan ***et al.*** (1964) were used to refer to the different chromosomes; that is, ratio 1.0 to <1.7 was "m" (metacentric) chromosome, 1.7 to <3.0 "sm"(sub metacentric) chromosome, 3.0 to <7.0 "st"(sub telocentric) chromosome and >7.0 "t" (telocentric) chromosome.

Results

Procedure related observations

Maximum number of dividing cells occurred when the samples were collected from day old larvae of ***O. pabda***. The fixative acetic alcohol (1:3) was found suitable for the present material. Colchicine (0.05%) treatment for four hours and one day old larvae gave satisfactory results in respect of degree of contraction of the chromosomes and frequency of prometaphase plates. Variations in larvae age and colchicine treatment duration were investigated, which gave no better results (Figs. 1 and 2) Hydrolysis of the body tissues in 10% HCl for 10 minutes at 60^0C was found adequate to soften the specimen. Mordanting of the hydrolysed tissues for 6 minutes in 2% iron alum solution and staining for 10 minutes in 0.5% haematoxylin produced satisfactory degree of staining of the chromosomes.

General observations on the chromosome complement

Present investigation revealed that the somatic chromosome number of ***O. pabda*** was 2n = 42 (Fig. 3). No deviation of chromosome number was detected. However, utmost care was taken in selecting undistorted plates for chromosome counting, since breakage of cell was frequent which could result in loss or gain of chromosomes from adjacent cells. Thus counting in such cells would obviously produce imperfect result. Of the 42 chromosomes, 18 chromosomes could be measured-which consisted of 10 metacentric, 7 sub metacentric and 1 sub telocentric chromosomes (Table. 1). For easy reference, chromosomes in the photographic prints were numbered arbitrarily. Based on microscopic observations and/or respective measurements it was presumed that chromosome number 1 & 6, 11 & 25, 10 & 36 and 14 & 35 of the complement shown in Fig. 3 were homologous pairs. However, it could not be ascertained with certainty because of limitations of time and materials.

Of the 18 chromosomes that could be measured the longest one- chromosome 1 was 3.9μ long having arm ratio 1.2, and the smallest one- chromosome 8 was 1.1μ long having arm ratio 1.2

Table 1: Measurements of selected chromosomes of the complement shown in Fig. 3 of *O. pabda*

Chromosome No. (as given in the Plate 3)	Length of Long arm (μ)	Short arm (μ)	Total length μ	Arm ratio L/S
1	2.1	1.8	3.9	1.2
3	1.0	0.8	1.8	1.3
5	1.1	0.6	1.7	1.8
6	1.8	1.2	3.0	1.5
7	1.2	0.9	2.1	1.3
8	0.6	0.5	1.1	1.2
9	0.8	0.5	1.3	1.6
10	1.3	0.9	2.2	1.4
14	1.3	0.6	1.9	2.2
16	1.0	0.5	1.5	2.0
17	1.0	0.4	1.4	2.5
18	1.3	0.8	2.1	1.6
25	1.2	1.2	2.4	1.0
26	1.6	0.5	2.1	3.2
31	1.2	0.6	1.8	2.0
35	1.3	0.6	1.9	2.2
36	1.3	0.9	2.2	1.4
42	1.3	0.6	1.9	2.2

Fig. 1: Late prophase stage in the body cells of day-old *Ompok pabda* larvae X 3800. Chromosome outlines are diffused and intermingled

Fig. 2: Over contracted metaphase chromosomes in the body cels of day-old *Ompok pabda* larvae unsuitable for chromosome measurements. X 3800

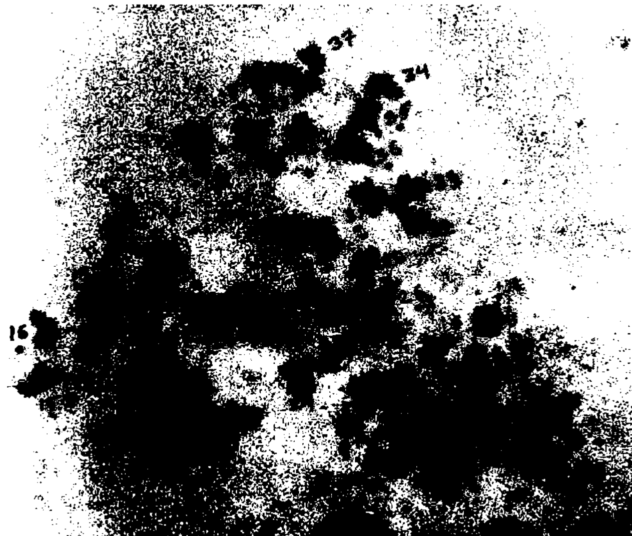

Fig. 3: Representative somatic complement in day-old *Ompok pabda* larvae selected for chromosome measurement. X 3800

(Table 1). The chromosomes 11 & 25 seemed to be the satellited pair because of the presence of prominent secondary constriction (Fig. 3).

Discussion

The present finding of 2n = 42 chromosomes in *O. pabda* is in agreement with the single earlier report of Rahman *et al.* (1995). Non-availability of more reports on chromosomal studies

in ***O. pabda*** and fishes in general could be related to considerable difficulties encountered in staining and slide preparation using appropriate fish larval tissues. Cells tend to suffer breakage easily causing unavailability of intact chromosome complements in higher numbers. Besides the small size higher number of chromosome is further disadvantageous for cytological studies. Although some chromosome, of this complement could be measured in the present study, it is worthwhile to perform further work to gain more information in the complete chromosome complement of ***O. pabda***.

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