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Chromosome analysis of Reba carp, *Cirrhinus reba*

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Abstract. Chromosome analysis is useful in different cytogenetics and genomics studies for various purposes e.g., linkage analysis, evolutionary studies and species identification and classification. Metaphase squash method using colchicine has been used for long time primarily for identifying chromosome number for many fishes from larval stage. Although majority of the Indian major carps have undergone such investigation, *Cirrhinus reba*, an important minor carp of Bangladesh is yet to be investigated. We investigated into the chromosome number and an effective concentration of colchicine solution along with duration of treatment in this study. Exposure to both 0.03% and 0.05 % colchicine for the duration of 6 and 4 h produced good quality chromosome spreads. The chromosome number obtained in the diploid (2n) individuals in this study was 50 which is very similar to other *Cirrhinus* species. The present results suggest basic information of chromosome number on which furtherinvestigation including detail karyotype analysis of*C. reba* and other related fish species can be done for cytogenetic studies.
Key words: *Cirrhinus reba*, Chromosome, Metaphase,Colchicine.

Introduction

Chromosomes are thread-like structures located inside the nucleus of animal and plant cells, and are composed of protein and the deoxyribonucleic acid (DNA). Genes embedded into chromosome pass from parents to offspring and contain the specific instructions that make each type of living creature in the universe (Cooper 2000).During mitosis and meiosis, the chromosome becomes condensed, to be organized and separated. In prokaryotes, there is usually only a single ring-like or liner shape chromosome whereas eukaryotic organisms contain multiple chromosomes. Each chromosome carries the hereditary material or part of the genetic code necessary to produce an organism.

The structure of chromosomes helps the DNA remains tightly wrapped around the proteins and it is crucial that cells have the correct number of chromosomes in order to function properly (Alberts *et al.* 2002).The morphology of eukaryotic chromosomes can be studied during mitosis (Lodish *et al.* 2000). Each species existing in nature could be distinguished by its specific number of chromosomes and is the recent way of classification. To date, a variety of chromosome analysis techniques such as tissue cultures (Fan and Fox 1990, Lomax *et al.* 2000), squashing techniques (Armstrong and Jones 2003), and cell suspensions of the tissues undergoing mitosis (Fan and

Fox 1990, Henegariu *et al*. 2001) have been developed to visualize chromosomes of fish at different developmental stages. In conventional chromosome preparation protocols, a spindle poison (e.g. colchicine) is employed to arrest the cells at their metaphase stage (Kligerman and Bloom 1977, Kligerman 1982). Since beginning, there were two methods for describing the morphology of chromosomes, one old method was configuration of chromosome at anaphase and second method, presently in use is the configuration at metaphase stage. We had an attempt to analyze the chromosome number of Reba carp, *Cirrhinus reba* in this study using metaphase staining method.

The reba carp belongs to the family Cyprinidae and native to Bangladesh, India, Nepal, and Pakistan (Dahanukar 2011). It is a popular table fish having high nutritional value (Gupta 1975, Afroz 2014). The protein, fat and carbohydrate calories of reba are relatively higher than those in the Indian major carps (Khawaja 1966, Sharma and Simlot 1971). This species was documented as vulnerable in Bangladesh (IUCN 2000) as well as in India (Camp 1998) but recently reported as least concern under IUCN Red list of threatened species (IUCN 2015). Although considerable research has been conducted on different aspects of this species including feeding, breeding and reproductive biology but so far, no report is available on the chromosome analysis of this species. The objective of the present study was therefore analyzing the chromosomes of reba carp from Bangladesh waters for basic understanding of its chromosome number to be used in further cytogenetics or molecular studies.

Materials and Methods

One-day old larvae of *Cirrhinus reba* were collected from the Fisheries Faculty Field Laboratory Complex, Bangladesh Agricultural University and Bangladesh Fisheries Research Institute, Mymensingh. Larvae were incubated in four concentration of colchicine solution of 0.02, 0.03, 0.04 and 0.05% for four different durations of (2, 4, 5 and 6 h) at room temperature. The procedure was followed as described by Shao *et al*. (2010). After collecting from hatchery, the larvae were placed in petridish to swim containing different concentrated colchicine solutions. Then the larvae were placed in 0.7% NaCl for 20 min and then in fixative solution (methanol: acetic acid = 3:1) for 30 min. The embryonic tissues were placed in a 5 mm diameter and 7 mm deep flat-bottomed hole of Perspex block with 2-3 drops of 50% acetic acid. Tissues were ground for 1 min using a 3 mm diameter glass rod. Ten min after grinding, the cell suspensions were taken up into a capillary tube and dropped from 36-50 cm height on to a clean glass slide placed on a hot plate (45^{o}C) and 2-3 circles were prepared. Slides were incubated in freshly prepared 10% Giemsa solution for 20 min. Then the slides were washed with xylene to remove the staining synthetic chemicals. Slides were mounted with DPX and finally number of chromosomes was observed and counted under a compound microscope (Olympus CKX 41) using Plan Achromat 100x objective lens, with immersion oil, in metaphase stage.

Results and Discussion

Observation of chromosome spreads conducted by metaphase squashing method using four different concentration of colchicine solution (0.02, 0.03, 0.04 and 0.05%) was rated manually on the basis of visibility (Table I, Fig. 1). A total of eight combinations of colchicine concentrations and incubation durations were tested. Among these two combinations, 0.05% colchicine for 4 hrs and 0.03% colchicine for 6 hrs gave excellent results in term of chromosome spread quality (Table I). Chromosome photomicrography of the selected plates revealed that somatic chromosome number of *C. reba* was 50 (2n) (Figs. 1 & 2).

Table I. Arbitrary grading of metaphase-chromosome spread observed with different concentration of colchicine at various durations

Sample type	Colchicine solution (%)	Duration (h)	Comments
01	0.05	5	+
02	0.05	4	++
03	0.05	2	-
04	0.04	5	+
05	0.04	4	+
06	0.03	6	++
07	0.02	5	-
08	0.02	4	-

++ Good, + Moderate, –Not good

Analysis of chromosome is important for a number of reasons in cytogenetics and genomics research e.g., exploring linkage groups by corresponding chromosome count, enumerating ploidy for natural or engineered organism, identify physical and genetic map for quantitative trait loci studies for important traits (sex-linkage for example), determining genome length and identifying DNA markers flanking to different linkage groups (i.e., chromosomes), classifying species and so on.

Among various techniques used to visualize the chromosomes of fish at different developmental stages, squash technique has been a simple method for spreading and flattening metaphase chromosomes (Mirzaghaderi 2010). However, air drying method is the most widely adopted method developed for preparing animal chromosomes. In chromosome preparation method, a spindle poison (i.e. colchicine) is employed to arrest the cells at their metaphase stage by preventing progress to anaphase.

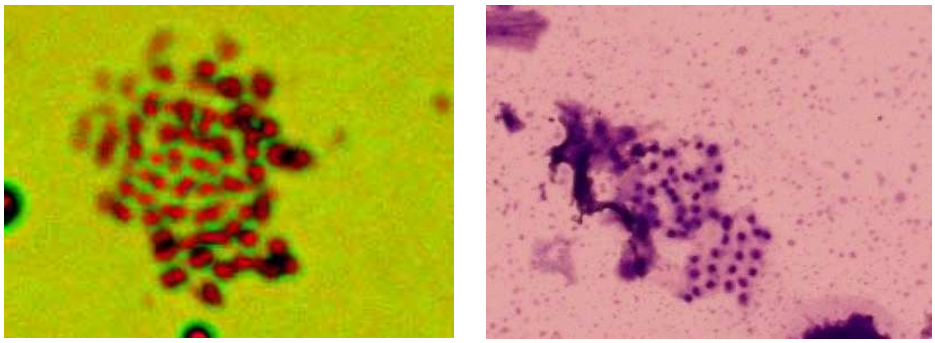

Fig. 1 (a) Fig. 1 (b)

Fig. 1. Metaphase chromosome spreads (100x) of diploid cells of *C. reba* treated with colchicine at 0.05% concentration for 4 h (a) and at 0.03% concentration for 6 h (b).

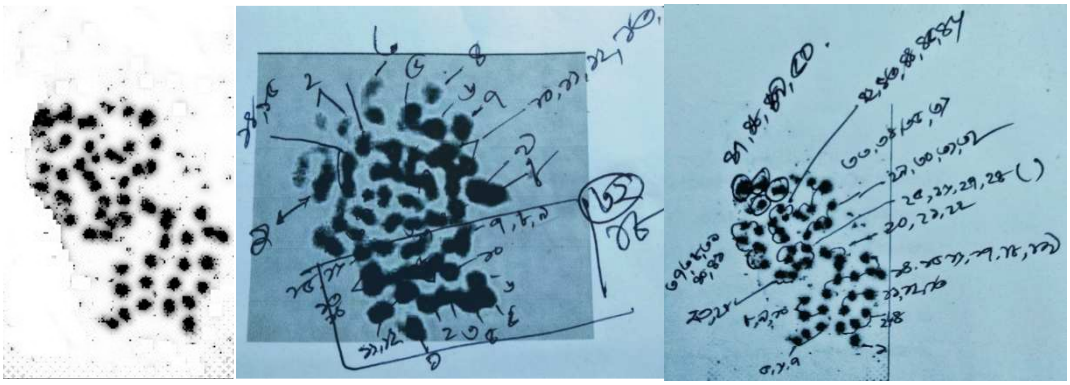

Fig. 2. Chromosome count of diploid cell spreads of *C. reba* (2n = 50).

Metaphase chromosome spreads of a diploid *C. reba* provided better visualization at 0.03% colchicine for 6 hours and 0.05% colchicine concentration for 4 hours with 10% Giemsa staining for 20 min in this study. Some of the previous works (e.g., Hussain *et al*. 1991, Pradeep and Zain 2011; Shao *et al*. 2010) employed similar concentrations (10%) of staining but with a lower incubation period. Mahfuz *et al*. (2014) used colchicine (0.05%) treatment for 2, 2.5 and 3 h using two day-old larvae of *L. rohita* Slide preparations were done following hydrolysis (10% HCl), mordanting (2% iron alum) and staining with haematoxylin. Colchicine treatment for 2 h gave satisfactory results in respect of degree of contraction of the chromosomes. In our experiment, we suggest 0.03% colchicine and 4 hours incubation should be set as a minimum requirement for better visualization of chromosome spreads. To achieve clear and identifiable metaphase chromosome spreads, it is essential to select the proper concentration and the correct incubation period of the poison (Rieder and Palazzo 1992). While insufficient concentration and/or duration of exposure to the spindle poisons fails to arrest the cells at the metaphase stage, very high

concentrations and/or overly long exposure duration may lead to chromosomal condensation (Rieder and Palazzo 1992; Wood *et al*. 2001). Besides these, a number of factors may inhibit the process, for example, age of the larvae, improper grinding of the tissue, amount of cell suspension dispersed onto the slide etc. apart from the colchicine concentrations.

There are great variations in somatic chromosomes of fishes. The diploid number in fishes ranges from 12 or 16 to 239+7 (Havelka *et al.* 2016). The highest number of 239 has been recorded in Adriatic sturgeon *Acipenser naccarii* as reported by Fontana and Colombo (1974). Chromosome number variation has also been seen. In *Lepidocephalichthys guntea* diploid number of 52 chromosomes in male and 51 in the female were observed (Sharma *et al.* 2002). They reported that one telocentric chromosome being less in females. Reviews on chromosome studies (Ojima 1985, Manna and Das 1994) showed majority of families of fishes have chromosome number in the range of 2N= 44-52 with predominantly acrocentric or sub-telocentric chromosomes. However, in modal number of diploid chromosome is 2N= 48. In about 138 species the peak was 2N= 46. In about 238 species it was noticed that the peak was 2N= 50. These numbers are counted on well spread metaphase stage and based upon colchicine citrate dry method.

In Cyprinidae family (wherein *C. reba* belongs), chromosome numbers were found to be varied from 48-50. In about 8 species of *Labeo,* 50 chromosomes were found but in *Labeo cerulaeus*, 48 chromosomes were obtained. Nayyar (1962) reported 2N=54 rod-like chromosomes in *Labeo dero* but Khuda-Bukhsh and Nayak (1982) found 2N= 50 with a large number of biarmed chromosomes in this species. In *Labeo rohita*, the 2n number of chromosomes is 50, out of which metacentric chromosomes are 18, the sub-metacentric chromosomes are 8, and sub-telocentric are 24. Similarly, another important major carp is *Gibelion catla*, which have the same number with distinguishable 8 metacentric, 16 sub-metacentric and 26 sub-telocentric. *Cirrhinus cirrhosus* has 6 metacentric, 26 sub-metacentric and 18 sub-telocentric amounting the total 50. Our investigation reveals 2N=50 for *C. reba* indicating of closeness of species in Cyprinid group. However, we could not distinguish the chromosomes on the basis of centromere position in this study. Chromosome numbers of 70% of the examined Cyprinids have been found to be 25 pairs (Khuda-Bukhsh 1982).

Chromosome analysis can be used for the genetic improvement of any organism (Al-Sabti and Metcalfe 1995; Sofy *et al.* 2008) by providing basic information on fish breeding programs such as inter-specific hybridization, chromosome manipulation techniques (Thorgaard and Disney 1990; Thresher *et al.* 2013), and genetic improvement of commercial fish stocks (Gui and Zhu 2012). Nevertheless, methods targeting embryonic and larval stages of fish have faced difficulties in achieving a reliable number of identifiable and wide-spread metaphase chromosomes probably due to the variations in mitotic cell division rates among different fish species (Shao *et*

al. 2010). We indicate further karyotyping studies on this species for comprehensive understanding of chromosome structure (including length and centromeric position).

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