

## ESTIMATION OF PLOIDY AND HYBRIDIZATION STATUS BY ERYTHROCYTE AND NUCLEUS SIZE ANALYSIS IN CARPS

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### ABSTRACT

The present experiment was designed to observe whether the nuclear volume and area are affected by the ploidy and hybrid status of the individual. Polyploidy was induced by heat shock treatment given at  $44 \pm 0.5^{\circ}\text{C}$  for 30 seconds and 45 seconds which was found to be most effective (64.7%) for induction of triploidy in *Cyprinus carpio*. Cell and nuclear volume and cell and nuclear area varied significantly in triploid fishes as compared to those of controls. Triploid fishes showed significantly higher growth compared to diploid counterparts. It was also observed that catla x rohu hybrid and its parents showed significant difference in the nuclear volume and area of their erythrocytes. Except nuclear volume, all the parameters were significantly different between catla and catla x rohu hybrid. The hybrids showed a closer relationship with catla as compared to rohu.

**Key words:** Ploidy, hybridization, nucleus size

### INTRODUCTION

Genetic manipulation through hybridization (Chaudhary, 1959), ploidy induction (Thorgaard, 1986), and chromosome and gene manipulation has been used to improve the somatic and reproductive performance of commercially important fishes. Because of the additive genetic effect, economically important characters are enhanced and the individuals perform better under the optimum environmental conditions. Inter-generic hybrids like catla x rohu (Chaudhary, 1971; Khan, 1991) and triploids of tilapia (Varadaraj and Pandian, 1988) have been a success in the past. Instances of naturally occurring hybrids have also been reported

(Natrajan *et al.*, 1976) because of the common breeding grounds of these individuals and polyploids (Kirpichnikov, 1981).

However, the genetic identification of hybrids and polyploid stock has been found to be difficult. Although many direct and indirect methods like flow cytometry (Magee and Philipp, 1982), chromosome counting (Beck *et al.*, 1980), nucleic acid quantification (Allen, 1983) and many more methods are available, a simple and easier method was adopted here. Based on this method, the nucleic acid contents analysis was carried out by measuring the size and

volume of erythrocytes and their nuclei. The size of erythrocytes has got direct relationship with genetically altered polyploids and hybrids (Vejaratpimol and Pewnim, 1990).

#### MATERIAL AND METHODS

For ploidy induction studies, the fertilized eggs of *Cyprinus carpio* were subjected to heat shock at different temperatures for 30 and 45 seconds (Table 1). After a period of two months, blood smears were prepared from control and test groups of fishes.

For the identification of hybrids and their parents, rohu, catla and their hybrids

were procured from Powarkheda Fish Farm. The blood smears were prepared from 10 randomly selected fishes of each category, fixed in methanol and stained with Giemsa (10%). The area and volume of erythrocytes and their nuclei were estimated with the following formulae :

$$\text{Area} = a b / 4$$

$$\text{Volume} = a^2 b / 1.91$$

$$\text{Where } a = \text{minor axis}$$

$$b = \text{major axis}$$

Chromosomal plates were prepared to confirm the ploidy status of the fishes (Reddy and John, 1986). The estimated values of nuclear and cell volumes, and area were recorded and subjected to statistical analysis .

**Table 1 : Heat shock treatment for triploidy Induction in *Cyprinus carpio***

Treatment Temp. (°C)	Duration (sec)	Hatching (%)	Survival (%)	Results
38±0.5	30	69.66	54.83	Diploid
	45	79.66	51.06	Diploid
40±0.5	30	75.32	55.17	Diploid
	45	77.91	71.64	Diploid
42.±0.5	30	88.19	83.80	Diploid
	45	94.97	71.18	Diploid
44±0.5	30	18.47	70.58	Triploid
	45	45.00	61.11	Triploid
46±0.5	30	32.91	—	—
	45	39.08	11.76	Triploid
CONTROL GROUP		83.52	61.18	Diploid

**RESULTS AND DISCUSSION**

Somatic growth was found to be 12.5% more for length and 30.5% more for weight after 75 days of rearing of triploidy induced *C. carpio* (Table 2). About 64.7 and 33.3% triploids were produced at  $44 \pm 0.5$  and  $46 \pm$

compared, it was observed that cell and nuclear area were highly significant ( $P < 0.01\%$ ) while the cell and nuclear volume were significantly different ( $P < 0.05\%$ ). Critical difference test showed that control group has got highly significant difference

**Table 2 : Average growth parameters of triploids of *C. carpio* in comparison to diploid fishes**

Temperature (°C)	Length (cm)			Weight (g)		
	Age (d)			Age (d)		
	15	45	75	15	45	75
Control	0.6	0.9	3.2	.075	.199	.383
44±0.5	0.8	1.45	3.8	.085	.312	.559
46±0.5	0.75	1.35	4.2	.085	.298	.524

0.5°C temperatures. The values of the parameters studied are given in Table 3. When triploid and diploid individuals were

( $P < 0.01\%$ ) from the two test groups (Table 3A, 3B).

Different chromosome numbers have

**Table 3 : Cellular and nuclear volume and area of diploid and triploid *C. Carpio***

Temperature (°C)	Volume ( $\mu\text{m}^3$ )		Area ( $\mu\text{m}^3$ )		Triploidy induced (%)
	Cell	Nucleus	Cell	Nucleus	
Control	3.342 <sup>-04</sup>	4.630 <sup>-05</sup>	6.672 <sup>-03</sup>	1.792 <sup>-03</sup>	—
44	4.057 <sup>-04</sup>	6.404 <sup>-05</sup>	7.717 <sup>-03</sup>	2.186 <sup>-03</sup>	64.7%
46	4.608 <sup>-04</sup>	6.663 <sup>-05</sup>	8.287 <sup>-03</sup>	2.141 <sup>-03</sup>	33.3%

**Table 3A : Analysis of variance for volume and area of erythrocyte and its nucleus in *C. carpio***

Source of variation	Degrees of freedom	Volume		Area	
		Cell	Nucleus	Cell	Nucleus
Between Species	3-1=2	5.558*	7.93*	9.92**	12.08**
Error	15-2=13				
Total	16-1=15				

**Table 3B: CD test for volume and area of erythrocyte and its nucleus in *C. carpio***

CD value for cell volume	= 0.367
CD value for nuclear volume	= 0.764
CD value for cell area	= 0.419
CD value for nuclear area	= 0.131

Temperature	Volume		Area	
	Cell	Nucleus	Cell	Nucleus
Control & 44± 0.5°C	0.625**	1.775**	1.045**	0.394**
Control & 46± 0.5°C	1.176**	2.036**	1.615**	0.349**

(\* Significant at 5% , \*\* Significant at 1%)

been reported in *C. carpio* by different workers (Blaxhall, 1983); the diploid number was observed to be  $98 \pm 2$ , while the triploid number was  $140 \pm 2$ . Although many methods are available to differentiate the genetic status of the individuals, the present method of erythrocyte size and nuclear volume used here is quick and more reliable (Cimino, 1973; Wolter *et al.*, 1982; Vejaratpimol and Pewnim, 1990).

Based on erythrocyte volume, Benfey and Sutterlin (1984) have sorted out the ploidy-induced fishes. Differences in

erythrocyte volume of diploid and triploid *C. macrocephalus* were recorded to be appreciable and identifiable (Vejaratpimol and Pewnin, 1990). In the present also, the described parameters indicated significant variation between the test and control group of fishes.

The average values for volume and area of cell and nucleus of catla x rohu hybrid and its parents are given in Table 4. The test of significance shows that these values for catla x rohu hybrid are significantly higher ( $P < 0.01$ ) than parents (Table 4A and 4B).

**Table 4: Cellular and nuclear volume and area of erythrocyte of *L. rohita*, *C. catla* and their hybrid**

Species	Volume ( $\pm$ S.E.) ( $\mu\text{m}^3$ )		Area ( $\pm$ S.E.) ( $\mu\text{m}^2$ )	
	Cell	Nucleus	Cell	Nucleus
<i>L. rohita</i>	2.674±0.001 <sup>-04</sup>	2.788±0.002 <sup>-05</sup>	5.878±0.002 <sup>-03</sup>	1.287±0.002 <sup>-03</sup>
<i>C. catla</i>	3.365±0.002 <sup>-04</sup>	3.512±0.002 <sup>-05</sup>	6.627±0.001 <sup>-03</sup>	1.468±0.003 <sup>-03</sup>
Catla X rohu hybrid	3.571±0.002 <sup>-04</sup>	3.581±0.001 <sup>-05</sup>	6.997±0.003 <sup>-03</sup>	1.500±0.001 <sup>-03</sup>

**Table 4A :** Analysis of variance for volume and area of erythrocyte and its nucleus in *L. rohita*, *C. catla* and their hybrid

Source of variation	Degrees of freedom	Volume		Area	
		Cell	Nucleus	Cell	Nucleus
Between Species	3-1=2	24.36**	17.286**	18.322**	14.555**
Error	29-2=27				
Total	30-1=29				

**Table 4B :** CD test for volume and area of erythrocyte and its nucleus in *L. rohita*, *C. catla* and their hybrid

CD Values for cell volume	= 0.170
CD Values for nuclear volume	= 0.187
CD Values for cell area	= 0.236
CD Values for nuclear area	= 0.051

	Volume		Area	
	Cell	Nucleus	Cell	Nucleus
1. Rohu & catla	0.688**	0.724**	0.747**	0.18**
2. Rohu & hybrid	0.895**	0.793**	1.118**	0.212**
3. Catla & hybrid	0.207*	0.069	0.371*	0.032*

(\* Significant at 5% , \*\* Significant at 1%)

For catla x rohu hybrid, all the parameters were significantly different from rohu ( $P < 0.01$ ). Similarly except for nuclear volume, the hybrid showed significant difference from catla. Both the parents had significant differences among themselves. The erythrocytes, and nuclear size and volume of hybrid showed a tendency of leaning towards its male parent (catla).

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