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Verification of Triploid Golden Mahseer (*Tor putitora*) by Erythrocyte Measurement

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Abstract

The present study was focused on the conformity of the triploidy induction in golden mahseer. Erythrocyte measurement which is an indirect method for the conformity of triploidy induction in fish was followed for this purpose. Triploidy induction was carried out by pressure shock and thermal shock after 12 min of post-fertilization. The recorded results reflected 24.63% larger average size of erythrocytes cell in triploids (13.51 ± 1.13 μm) over the diploids (10.84 ± 1.01 μm). Similarly, the average measurement size of nucleus was recorded 21.27% lager in triploids (6.1 ± 0.61 μm) than the diploid counterpart (5.03 ± 0.42 μm). The size of erythrocyte cell was measured in the range between 8.4 and 13.28 μm for diploid and 10.4–15.39 μm for triploid while size of nucleus was recorded as for 4.24–6.12 μm for diploid and 4.73–7.37 μm for triploids, respectively. There was significant variation ($P < 0.05$) in major axis of erythrocytes cell and nucleus both in diploid and triploids individuals. Hence, the study showed the applicability measurement of erythrocyte cell as an indirect method for triploidy conformity in fish.

Keywords: Erythrocyte measurement, Golden mahseer, Nucleus, Triploids

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Introduction

Golden mahseer is an important indigenous fish species of Indian Himalayan region and supports the livelihood, provides nutrition security as a protein-rich food to the people dwelling in hills. Although, the aquaculture of this species is not in practice due to the slow growth, the wild catch of the fish contributes major share to the fish eaters in hills. The slow somatic growth is the major constraint for its aquaculture establishment and enhancing aqua-tourism of this fish. Sexual maturation is an energetically expensive stage in the life cycle of most fishes, resulting in reduced growth, decreased flesh quality, and increased susceptibility to disease.[1] Nowadays, production of sterile fish by ploidy manipulation is a feasible technology for achieving a better somatic growth and resistance against diseases. Triploidy induction is one of the most tested and successful techniques and considered one of the best ways of producing sterile fishes.[2,3] The extra genetic material in triploid fish supposed to be useful for increasing growth and maintaining heterozygosity. Consequently, it also improves the flesh quality of the fish, reduces mortality, and prevents fish reproduction, thereby minimizing the possible impact of genetic and ecological disorder linked to the interactions between wild and cultured fishes.[4] Triploidy refers to such a condition where three chromosome sets occur in the nucleus of individual organism, including fish. Triploidy is characterized by the change in normal diploid (2n) set of chromosomes to the state of triploid (3n) with an additional set of chromosomes.[5-8] Induction of triploidy can be achieved by applying sublethal treatments to newly fertilized eggs with the help of heat shock[9] or cold shock,[10] pressure shock,[11] electric shock,[12] and chemical exposure.[13] All techniques are based on the concept of suppression of second polar body by any kind of shock or treatment.[2,14,15] There are no reports from India concerning induction of triploid in golden mahseer (*Tor putitora*). The initial reports of trials on ploidy induction for the production of triploid fish were carried out in the forties.[16,17] The verification of triploids is an important step in triploidy induction process, which has mainly depended on direct method of chromosome count[18,19] which is an expensive and time taking method. In spite of this, indirect methods such as erythrocyte size measuring and counting of nucleoli are a few of most feasible indirect methods for ploidy identification. In the previous studies counting of nucleoli,[20] measurement of nuclear and cellular size of erythrocytes[21] are considered for the detection of ploidy confirmation. Measurement of the erythrocyte cell and nucleus size[22] and flow cytometry[23,24] is easy and more applicable in field conditions. The size of the erythrocyte cells and nucleus in triploids remains relatively larger in comparison to the diploids, however, the cytoplasm and the nucleus ratio remain constant.[3,25] Therefore, in this study, indirect verification of triploid golden mahseer was carried out by erythrocyte cell and nucleus measurement on the blood smear obtained from diploids and triploids individuals.

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Materials and Methods

The study was carried out at ICAR-Directorate of Coldwater Fisheries Research (ICAR-DCFR), Bhimtal (latitude 29^0 21'N,

longitude 79^0 34'E, 1370 masl), Uttarakhand. Blood smear was prepared by taking the blood drop on clean slide by cutting off caudal peduncles of 5-month-old fingerlings from both the batches for indirect triploidy verification. Triploidy induction was carried out by pressure shock and thermal shock after 12 min of post-fertilization. Blood samples were taken from the fingerlings of diploid and triploid stock and smear was prepared by dropping a small drop of blood on a clean glass slide and spread using another slide as spreader. The spreader catches the drop when placed at an angle of 45° by capillary action along its edge, then by a quick and smooth motion, blood drop was stretched from one to the other end of the slide and prepares a smear on the slide. To avoid the deformation of the erythrocytes, first fixation (physical fixation) was done immediately by shaking the slide in the air. Panoptical Pappenheim method was carried out for the staining. Pink or red-violet color of the smear is the indication of correctly stained slide, while blue or violet color indicated that the smear was left for a too long period in contact with the dye. Then, the stained slides were gently washed with distilled water and left for air drying for 3–5 min before microscopic examination. The major axis of 50 erythrocyte's cell was measured for each specimen from dry blood smears with the aid of a microscope camera. Analysis of variance (ANOVA) with 5% significance level was done for statistical analysis.

Table 1: Erythrocyte cell and nucleus size (µm) difference in diploid and triploid golden mahseer (*Tor putitora*)

Avg. size (µm)	*Diploidy 2N*	*Triploidy 3N*
Erythrocyte cell	10.84±1.01	13.51±1.13
Nucleus	5.03±0.42	6.1±0.61

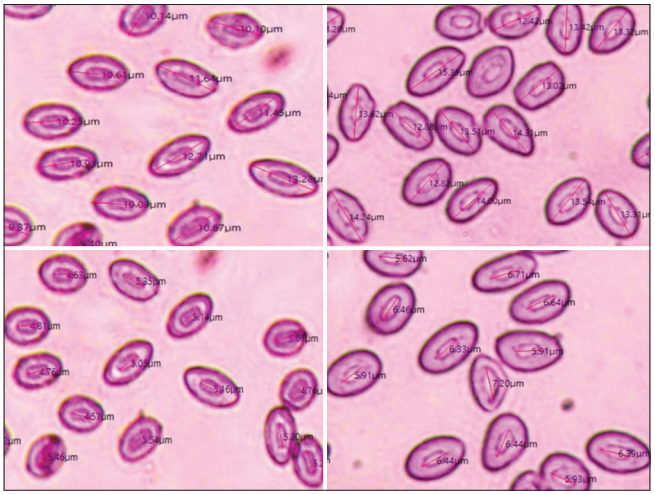

Figure 1: (a) Erythrocyte cell in diploids, (b) erythrocyte cell in triploids, (c) nucleus cell in diploids, (d) nucleus cell in triploids and of *Tor putitora* at 40×

Results and Discussion

Triploid cells have more DNA than diploid cells, their nuclei and the cells themselves are significantly larger than diploid nuclei and cells.[26] Kizak *et al.*[27] observed 29% larger erythrocyte size in triploid brow trout over the diploids. Similarly, the sizes of triploid *Puntius gonionotus* were reported approximately 1.63 times larger because of having extra chromosome set than diploid erythrocytes.[22] Espinosa *et al.*[28] reported 100% correctly identification of triploidy in *Oncorhynchus mykiss* by erythrocyte measurement. In the present study, the recorded results reflected 24.63% larger average size of erythrocytes cell in triploids (13.51 ± 1.13 µm) over the diploids (10.84 ± 1.01 µm). Similarly, the average measurement size of nucleus was recorded 21.27% lager in triploids (6.1 ± 0.61 µm) than the diploid counterpart (5.03 ± 0.42 µm). The size of erythrocyte cell was measured in the range between 8.4 and 13.28 µm for diploid and 10.4–15.39 µm for triploid while size of nucleus was recorded as for 4.24–6.12 µm for diploid and 4.73–7.37 µm for triploids, respectively (Table 1 and Figures 1 and 2).

The results of the present study revealed an increase in erythrocyte size in agreement with previously reported on triploid fish species such as turbot, catfish, sea trout, hybrid red tilapia, sea bass, and rainbow trout.[29-35] The erythrocyte nucleus major axis is already described by various researches as a distinct parameter for the ploidy identification in fish species.[36-40] Ioan *et al.*[41] described that the ratio between the cytoplasm and the nucleus remains constant; and if the volume of the nucleus increases, the cytoplasm volume will increase too and the cell will be larger in size. The data were further analyzed by (ANOVA). There was considerable variation in major axis of erythrocytes cell and nucleus both in diploid and triploids individuals. The study data reflected a significant difference ($P < 0.05$) in size measurement of erythrocyte and nucleus cells of diploids and triploids.

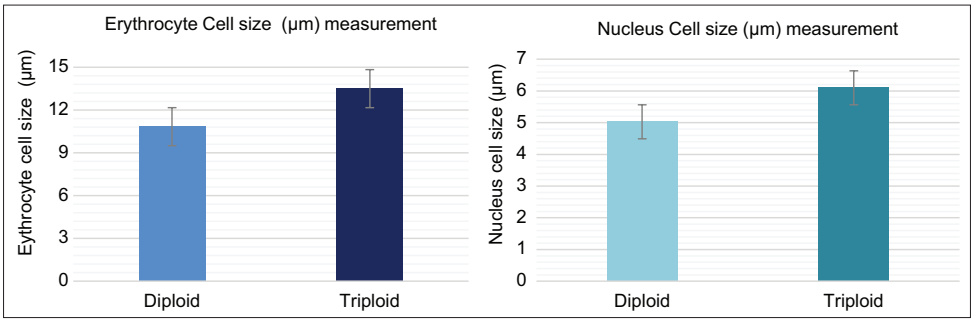

Figure 2: Erythrocyte cell and nucleus measurement in diploid and triploid *Tor putitora*

Conclusion

A significant difference in erythrocyte cells and nucleus measurement was observed for diploid (control) and triploid individuals. Hence, study reveals that this indirect method is feasible and easier as compare to expensive and time-consuming karyotyping method for ploidy identification in fish.

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