Induction of triploidy with caffeine treatment in the African catfish (*Clarias gariepinus*)

Turan, F.*; Guragac, R.

Received: February 2014 Accepted: December 2014

Abstract

Induction of triploidy is one of the biotechnological methods in aquaculture used for genetic manipulation. It refers to a state where organisms have three complete sets of chromosomes instead of two and can result in sterility. Caffeine treatment that is safe and inexpensive, serve to induce triploidy in catfish. To suppress the second meiotic division, fertilized eggs were exposed to three different concentrations (5, 10 and 15 mM) of caffeine solution for 20 min beginning at 3 min after fertilization. After that, the eggs were incubated at ambient temperature until hatching. The induction of triploidy in fry was determined for three concentrations of caffeine by means of flow-cytometric analysis. The lowest rate of triploidy (20.40 ± 1.13%) was obtained in the group treated with 5 mM caffeine and the highest (69.10 ± 2.18%) in the group treated with 15 mM caffeine. Our results suggest that caffeine can be used to induce triploidy in catfish.

Keywords: *Clarias gariepinus*, Triploidy, Caffeine, Flow cytometry.

Faculty of Marine Science and Technology, University of Mustafa Kemal, P.O. Box: 31200 Hatay, Turkey

* Corresponding author's email: fturan@mku.edu.tr

Introduction

As a technique of chromosome engineering, triploidy is widely accepted method for producing sterile fish for aquaculture and fisheries management. Gonadal development and gametes production in fish may negatively affect growth and feed conversion rates that decrease the percentage of fillet or marketable production because during the gonadal development, the fish mobilizes part of the absorbed nutrients and/or part of the body reserves for gonadal development and gametes production (Purdom, 1983; Thorgaard, 1986; Henken *et al*., 1987). Therefore, production of sterile fish is useful for the aquaculture industry.

Triploidy induction for aquaculture has been applied to several species of salmon (Johnston *et al*., 1989), trout (Bonnet *et al*., 1999) and catfish (Hammed *et al*., 2010; Karami *et al*., 2010). African catfish (*C. gariepinus*) is one of the most important tropical cultured fish due to high growth rate, high stocking-density capacities, high consumer acceptability and high resistance to poor water quality and oxygen depletion (Akinwole and Faturoti, 2007; Adewolu *et al*., 2008).

For the mass production of triploid catfish in commercial hatcheries, large quantities of eggs must be treated simply, cheaply and safely because the treatment must not damage either humans or the natural environment. Cold (Sun *et al*., 1992; Yang *et al*., 1997) and hot (Pandian and Koteeswaran, 1998) treatments for triploid induction are safe because no chemicals are used. However, these treatments require an optimal treatment temperature to be maintained in a large volume in order to treat a large quantity of eggs. Pressure treatment is also considered to be safe (Pandian and Koteeswaran, 1998). However, in pressure treatment specific equipments are required and it is usually difficult to treat many eggs on a commercial scale. Chemical treatments such as cytochalasin B (CB) (Maldonado *et al*., 2001; Liu *et al*., 2004) and 6-dimethylaminopurine (6-DMAP) (Yan and Chen, 2002; Liu *et al*., 2004) are highly toxic, very expensive and not realistic for large scale treatment in commercial hatcheries. On the other hand, caffeine is a chemical recognized as a food constituent in many countries. Caffeine is safer and cheaper than either the CB or 6-DMAP.Therefore, caffeine is a promising agent for the mass production of triploids (Okumura *et al*., 2007). Nonetheless, triploid induction by caffeine treatment has been reported only in bivalves (Scarpa *et al*., 1994; Okumura *et al*., 2007) and trout (Turan *et al*., 2012a).In this study, we investigated the potential use of caffeine treatment for commercial production of triploid African catfish.

Material and methods

Wild *C. gariepinus* broodstock was captured from the Asiriver, Hatay, Turkey and transported to the Mustafa Kemal University Aquaculture Research Unit. The incubation tanks and caffeine solutions were already prepared prior to fertilization. The flow-through system was made to run and regulate. Proper aeration was ensured by the use of electric

airpumps. Trouttriploidy induction protocol (Turan *et al*., 2012a) were modified and used as a baseline to set the parameters (caffeine dosage) for this species. In order to suppress the second meiotic division, fertilized eggs were exposed to three different concentrations (5, 10 and 15mM) caffeine solution for 20 min beginning at 3min after fertilization (Table 1). Diploid controls not subjecting caffeine treatment were originated from the same parents. Each treatment and control groups were done in triplicate. The fertilization was carried out in water at a temperature of25.0±1.2°C. Total hatching was noticed after 36 h of incubation. After absorption of the yolk sac, catfish larvae were fed *Tubifex tubifex* (Muller) provided ad *libitum*. At ten days, the larvae were fed a trout diet (Aquamaks, Turkey: 48% protein, 18 % lipid) three times a day ad *libitum*.

At the end of the experiment (60 days after absorption of yolk sac), total length and weight were measured for each individual and survival rate were calculated for each treatment. The larvae were sampled from each treatment group at 3 day after hatching (d.p.h) and fixed with 70% ethanol and stored at -20°C until analysis (Nomura *et al*., 2004). For measurement of the relative DNA content, flow cytometry (FCM) was conducted using a BD FACS Canto flow cytometer (Beckton Dickinson Immunocytometry Systems San Jose, CA, USA). The method of FCM analysis was followed according to the protocol described by Çakmak **Yılmazer** (2011). The statistical testing to verify differences between the groups was carried out using a one-way analysis of variance (ANOVA).

Results

The triploid yield was ranged from 66.33±3.51% to 19.01±1.18% between the caffeine treatment groups (Table 1).

Table 1: Triploid rates in treated and control batches.

				Ploidy*	
Treatment	**Time after fertilization (min)**	**Shock duration (min)**	**Triploid Yield (%)**	**Percent Diploid (2n)**	**Percent Triploid (3n)**
Control	3	20	-	100	-
5 Mm	3	20	19.01±1.18	79.60	20.40±1.13
10 mM	3	20	38.50±0.73	58.28	41.72±0.80
15 mM	3	20	66.33±3.51	30.90	69.10±2.18

*Values (mean ± S.E. of triplicate) and total number of larvae (3 days after hatching) ploidies analyzed by flow cytometry.

The lowest rate of triploidy (20.40 ± 1.13%) was detected in 5 mM caffeine treatment at 25 °C for 20 min duration initiated 3 min after fertilization while the highest rate of triploidy (69.10 ± 2.18%) was observed in 15 mM caffeine treatment at 25 °C for 20 min duration initiated 3 min after fertilization (Table 1; Fig. 1).

These findings indicate that 15 mM caffeine was the best treatment for the induction of triploid catfish. The survival rate and weight gain of the caffeine treatment groups are shown in Table 2.

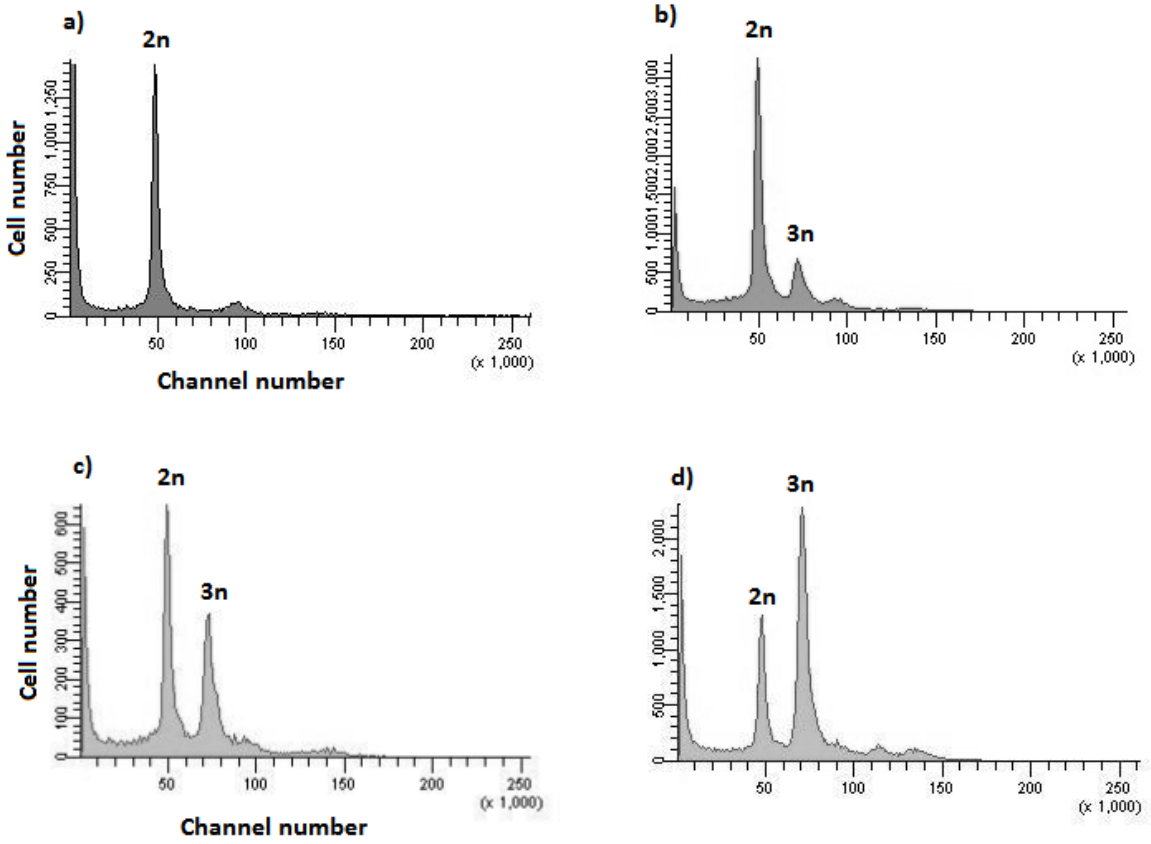

Figure 1: Flow cytometric histograms for the relative DNA content of somatic cells when somatic cell of normal diploid catfish are used as standard of normal diploidy. Diploid (2n) larva of control (a), triploid (3n) and diploid larva of treatedgroups (5 mM, 10 mMand 15 mM as b,c,d respectively).

Table 2: Survival rate and weight gain in treated and control batches.

Treatment	Time after fertilization (min)	Shock duration (min)	Survival rate (%)	Weight gain (g)
Control	3	20	74.16± 0.52^{b}	2.61 ±0.62^{a}
5 mM	3	20	72.58±0.45^{ab}	2.72 ±0.47^{a}
10 mM	3	20	70.49±0.76^{a}	2.40 ±0.73^{a}
15 mM	3	20	71.53±0.71^{a}	1.88 ±0.19^{a}

*Values (mean ± S.E. of triplicate) with same superscripts in each line indicate not significant differences ($p<0.05$).

The survival rates were ranged from 74.16% to 71.53%, and there was statistical difference between the experimental and control groups ($p<0.05$). However, no significant differences were detected in weight gain between the treatments and control group ($p>0.05$) (Table 2).

Discussion

The results of the present work have clearly demonstrated that caffeine treatment produce triploid African catfish. Until recently, there has been no published information on triploid induction by caffeine treatment in African catfish. The best triploid rate in African catfish was69.10 % in 15 mM caffeine treatment. This percentage may be low but strongly indicate that caffeine treatment on triploidization of catfish is effective. Richter *et al*.(1987) and Hammed *et al*. (2010) reported that the most effective timing and shock duration for triploid induction in African catfish is generally for 20 min duration initiated 3 min after fertilizationin African catfish. Therefore, we used similar timing and shock duration for triploid induction in present study. Turan *et al*. (2012a and 2012b) also reported the success of caffeine treatment on triploid production of rainbow trout. In the present study, increased caffeine concentration also increased the triploid rate. In order to obtain 100% triploid African catfish, optimizing caffeine concentration should be conducted in the future studies. In the present study, there was no adverse effect of the caffeine treatment on survival and weight gain of African catfish. Scarpa *et al*. (1994), Okumura *et al*. (2007) and Turan *et al*. (2012a) also reported similar results for the survival and weight gain in other species with caffeine treatments.

This is a first report to our knowledge regarding the potential of caffeine treatment on induction of triploidy in African catfish. The caffeine treatment is simple, safe and inexpensive. Therefore, this method can be applied for commercial production of triploid catfish in aquaculture. However, further investigation is required to obtain a 100% triploid African catfish population.

Acknowledgements

The study was supported by the project 0204 Y 0102 of University of Mustafa Kemal in Turkey. Also, the authors gratefully acknowledge the support of İzmir Institute of Technology (in Turkey) for the use of the Institute laboratory.

References

Adewolu, M.A., Adeniji, C.A. and Adejobi, A.B., 2008. Feed utilization, growth and survival of *Clarias gariepinus* (Burchell 1822) fingerlings cultured under different photoperiods. *Aquaculture*, 283, 64–67.

Akinwole, A.O. and Faturoti, E.O., 2007. Biological performance of African catfish (*Clarias gariepinus*) cultured in reticulating system in Ibadan. *Aquaculture Engineering*, 36,18–23.

Bonneta, **S., Haffrayb, P., Blancc**, **J.M., Valléec**, **F., Vauchezb**, **C., Fauréd**, **A. and Fauconneau, B., 1999.**Genetic variation in growth parameters until commercial size in diploid and triploid freshwater rainbow trout (*Oncorhynchus*

mykiss) and seawater brown trout (*Salmo trutta*). *Aquaculture*,173(1–4), 359–375.

Çakmak Yılmazer, Ö., 2011. DNA fragmentation, cell cycle analysis and Apotatik cell analysis (Annexin-V). I. cell death research techniques course. Dokuz Eylül University, İzmir, 223 - 232. ISBN, 978-975-441-349-6.

Hammed, A.M., Fashina-Bombata H.A. and Osinaike, A.O., 2010. The use of cold shock in inducing triploidy in African mud catfish (*Clarias gariepinus*). *African Journal of Biotechnology*, 9 (12), 1844-1847.

Henken, A.M., Brunink, A.M. and Richter, C.J.J., 1987. Differences in growth rate and feed utilization between diploid and triploid African catfish, *Clarias gariepinus. Aquaculture*,63 (1-4), 233-242.

Johnstone, R., Knott, R.M., McDonald, A.G. and Walsingham, M.V., 1989. Triploidy induction in recently fertilized Atlantic salmon ova using anesthetics. *Aquaculture*, 78, 229-236.

Karami, A., Christianus, A., Ishak, Z., Courtenay, S.C., Syed, M.A., Azlina, M. and Nooshinah, H., 2010. Effect of triploidzation on juvenile African catfish (*Clarias gariepinus*). *Aquaculture International*, 18(5), 851-858.

Liu, W., Heasman, M. and Simpson, R., 2004. Induction and evaluation of triploidy in the Australian blacklip abalone, *Haliotis rubra*: a preliminary study. *Aquaculture*, 233, 79–92.

Maldonado, R., Ibarra, A.M., Ramirez, J.L., Avila, S., Vazquez, J.E. and Badillo, L.M., 2001. Induction of triploidy in Pacific red abalon (*Haliotis rufescens*). *Journal of Shellfish Research.* 20, 1071–1075.

Nomura, K., Nakajıma, J., Ohta, H., Kagawa, H., Tanaka, H., Unuma, T., Yamauchı, K. and Araı, K., 2004. Induction of triploidy by heat shock in the Japanese eel *Anguilla japonica*. *Fisheries Science*,70, 247– 255.

Okumura, S., Araı, K., Harıgaya, Y., Eguchı, H., Sakaı, M., Senbokuya, H., Furukawa, S. and Yamamorı, K. , 2007. Highly efficient induction of triploid Pacific abalone *Haliotis discus hannai* by caffeine treatment. *Fisheries Science*, 73, 237- 243.

Pandian, T.J. and Koteeswaran R., 1998. Ploidy induction and sex control in fish. *Hydrobiology*, 384, 167–243.

Purdom,C.E., 1993. Genetic engineering by the manipulation of chromosomes, *Aquaculture*, 33, 287-300.

Richter, C.J.J., Henken, A.M., Eding, E.H., Van-Doesum, J.H. and DeBoer P., 1987. Induction of triploidy by cold-shocking eggs and performance of triploids of the African catfish, *Clarias gariepinus* (Burchell, 1822). In: Tiews, K. (Ed.), Selection, Hybridization and genetic engineering in aquaculture. Proceedings of the world symposium on selection hybridization and genetic engineering in aquaculture, vol.2, 27–30 May 1986, Berlin, Germany. *Schriften der Bundes for schungsantalt für. Fischeries Science*, 225–337.

Scarpa, J., Jorge, E.T. and Wada, K.T., 1994. Direct comparison of six methods to induce triploid in bivalves. *Aquaculture*, 119, 119–133.

Sun, Z., Song, Z., Li, N., Zhao, Y. and Guan, X.,1992. A preliminary study on the growth of triploid abalone (*Haliotis discus hannai* Ino). *Transaction of Oceanology and Limnology*, 4, 70–75.

Thorgaard, G.H., 1986. Ploidy manipulation and performance. *Aquaculture*, 57, 57–64.

Turan, F., Güragaç, R., Yigitarslan D. and Turan,C., 2012a. A preliminary study on induction of triploidy by caffeine treatment in the trout. First National Workshop on Marine Biotechnology and Genomics, Turkish Marine Research Foundation ISBN No 978-975-8825-28-8- Publication number, 36 204-211.

Turan F., Gürağaç R., Seyhan D. and Turan, C., 2012b. Triploidy induction by caffeine-thermal shock treatments in the trout. First National workshop on Marine Biotechnology and Genomics, Turkish Marine Research Foundation ISBN No 978-975-8825-28-8- Publication number, 36,212-217.

Yang H.S., Ting Y.Y. and Chen, H.C., 1997. Effect of cold shock on the production of triploid zygotes and the embryonic development of small abalone, Haliotis *diversicolor supertexta* Lischke. *Acta zoology Taiwan*, 8,67-78.